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Prevalence, risk factors, and geographical
distribution of zoonotic pathogens carried by
peri-urban wild dogs

Lana Claire Harriott
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Abstract

Established wildlife populations within peri-urban and urban environments provide an opportunity for the spread of zoonotic pathogens to and from human-associated environments. Wild dogs, including dingoes (*Canis lupus dingo*), feral domestic dogs (*Canis lupus familiaris*), and their cross breeds (hybrids) are common across the Australian mainland, and hybrid populations are particularly frequent within peri-urban environments. Previous studies have shown that wild dogs carry zoonotic pathogens of public health significance, however, data relating to peri-urban wild dogs has been limited to a few studies with a small geographical range and / or small sample size. To address the potential public health implications of peri-urban wild dogs, information regarding pathogen diversity, prevalence, risk factors and geographical distribution are required.

To investigate the diversity and prevalence of zoonotic pathogens in peri-urban wild dogs, a cross sectional survey was undertaken. Utilising council management programs and private trappers, 201 wild dogs were collected for necropsy. Analysis of faecal and blood samples indicated that helminth parasites were common, detected within 79.6 ± 5.4 % of wild dogs tested, however bacterial pathogens were much less prevalent. *Echinococcus granulosus* was the most prevalent parasite detected by necropsy, (prevalence 50.7 ± 6.9 %). Hookworms were found to comprise the second most common pathogen of zoonotic significance with an overall prevalence of 28.8% (± 7.1 %). Molecular characterisation identified the majority as *Ancylostoma caninum*. Several pathogens of zoonotic interest were not detected during the study, namely *Brucella suis* and *Neospora caninum*. This study provided essential baseline data on the prevalence of pathogens carried by peri-urban wild dog populations in north-eastern Australia, which is required to inform the management of wild dogs in peri-urban areas.

Characteristics of the most prevalent parasitic pathogens and their lifecycles suggested possible associations between the diet composition of wild dogs and their infection status. Whole stomachs were collected during necropsy and analysed for dietary composition and biomass. The majority of food items detected were mammalian prey species, most commonly: swamp wallaby (20.6 ± 6.08 %); canine species (prey) (10.6 ± 4.62 %); eastern grey kangaroo (10.0 ± 4.51 %); and deer species (10.0 ± 4.51 %). Wild dogs that consumed swamp wallaby (OR 1.79, $p < 0.05$) or unidentified species of macropods (OR 4.18, $p < 0.01$) were found to be significantly more likely to be infected with *E. granulosus* than dogs who had consumed non-macropod species. Wild dogs that had consumed various bird (OR 7.80, $p < 0.01$) or bandicoot species (OR 3.09, $p < 0.01$) were found to be significantly more likely to be infected with hookworm compared to dogs that had consumed

macropods. These findings suggest that diet composition is an important contributing factor to the infection status of wild dogs.

To explore the geographical distribution of pathogens and to determine the risk factors associated with their presence, spatial modelling techniques were applied to the two most significant (based on prevalence in wild dogs and their potential impact on human health) parasites detected within the study. Using model-based geostatistics our results indicated significant geographical variation in the probability of *E. granulosus* carriage, with a strong propensity for clustering. None of the modelled physical environment or climate variables were significantly associated with the presence of *E. granulosus*, however the fitted model accounted for the majority of the spatial dependence. In the case of hookworm, our results demonstrated that there was no significant spatial variation in the residual probability of hookworm carriage. A prediction map was generated using a *logit* back transformation of the final non-spatial multivariable model. Overlaying the predictive maps of *E. granulosus* and hookworm indicated that regions of high predicted hookworm prevalence overlapped with low to moderate predicted *E. granulosus* prevalence. These results suggest that due to the significant public health risks of *E. granulosus*, an integrated program that controls both parasites in the regions of high predicted hookworm prevalence would be beneficial. Regions of high predicted prevalence for *E. granulosus* correspond with regions of low predicted prevalence for hookworm. In this case, management programs could exclusively target *E. granulosus* to efficiently control the risk on human health.

This study describes the important role of wild dogs in the maintenance of zoonotic pathogens within peri-urban environments, identifies risk factors associated with pathogen carriage, and provides two predictive maps that have the potential to be used as spatial-decision supports tools to mitigate the public health risks of peri-urban wild dogs. The prediction maps will greatly assist land managers to best allocate their limited resources, and provide the opportunity for future research to assess the effectiveness of the applied control / management techniques on zoonotic pathogen prevalence in peri-urban wild dogs and their immediate habitat.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications during candidature

Journal articles

Cursino, M.S., Harriott, L., Allen, B.L., Gentle, M., Leung, L. K'P (2017) Do female dingo-dog hybrids breed like dingoes or dogs. *Australian Journal of Zoology* **65**, 112-119
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Conference abstracts

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Harriott, L., Gentle, M., Traub, R., Soares Magalhães, R.J., Cobbold, R. Helminth infections in peri-urban wild dogs and foxes. Australasian Society of Infectious Diseases, Zoonoses Conference, Brisbane, Australia. 25th – 26th July 2014.

Harriott, L., Gentle, M., Traub, R., Soares Magalhães, R.J., Cobbold, R. *Echinococcus granulosus* and other zoonotic pathogens of peri-urban wild dogs in south-east Queensland, Australia. European Vertebrate Pest Management Conference, Seville, Spain. 21st - 25th September 2015.

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Contributions by others to the thesis

The design and concept of this research was achieved through discussion with my advisory team including: Dr Rowland Cobbold; Dr Rebecca Traub; Dr Matthew Gentle and Dr Ricardo Soares Magalhaes. Collection of data was assisted by individual trappers and local governments across the study area. Dr Ricardo Soares Magalhaes assisted with the spatial analyses of the data. Tian Tian, a coursework Masters student conducted a small research project entitled ‘Prevalence and phylogenetic study of antimicrobial resistant isolates of *Escherichia coli*, *Salmonella spp.* and *Campylobacter spp.* from wild dog stomachs and faeces and their association with gender, age, location, date and diet’. A small part of the *E. coli* and multidrug resistance results have been included in Chapter 3. This thesis contributed towards a larger peri-urban wild dog project conducted by Biosecurity Queensland.

Statement of parts of the thesis submitted to qualify for the award of another degree

Tian Tian presented a report entitled ‘Prevalence and phylogenetic study of antimicrobial resistant isolates of *Escherichia coli*, *Salmonella spp.* and *Campylobacter spp.* from wild dog stomachs and faeces and their association with gender, age, location, date and diet’ which contributed to her completion of a coursework master’s degree in Veterinary Science with the University of Queensland. The project contributed towards a research component of the subject VETS7618.

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Wild dogs, Peri-urban, Zoonoses, Helminths, *E. granulosus*, Hookworm, Public Health, Ecology

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List of abbreviations used in Thesis

° C	Degrees Celcius
μL	Microliter
μm	Micrometre
ABS	Australian Bureau of Statistics
AK	Amikacin
AMC	Amoxicillin-clavulanate 2:1
AMP	Ampicillin
AMP-MCA	Ampicillin – MacConkey Agar
AUD	Australian Dollars
BGA	Brilliant Green Sulfa Agar
bp	Base Pairs
CAZ	Ceftazidime
CDC	Centre for Disease Control
CF	Compliment Fixation
CI	Confidence Interval
CIP	Ciprofloxacin
CN	Gentamicin
CT	Covert Toxocarosis
CVBD	Canine Vector Borne Disease
DNA	Deoxyribonucleic acid

EDTA	Ethylenediaminetetraacetic Acid
FFC	Florfenicol
FNQ	Far North Queensland
FOX	Cefoxitin
g	Grams
GB	Greater Brisbane
GIS	Geographical Information Systems
IACRC	Invasive Animals Cooperative Research centre
ITS	Internal Transcribed Spacer
IUCN	International Union for Conservation of Nature
KF	Cephalothin
LGA	Local Government Area
MCA	MacConkey Agar
MDR	Multi Drug Resistant
MH	Mueller Hinton Agar
mg	Milligrams
mL	Millilitre
mm	Millimetre
MRSA	Methicillin Resistant Staphylococcus Aureus
MSSA	Methicillin Sensitive Staphylococcus Aureus
NaCl	Sodium Chloride

NB	North Brisbane
ng	Nanograms
NSW	New South Wales
NT	Neurological Toxocarosis
OLM	Ocular Larva Migrans
PCR	Polymerase Chain Reaction
PBS	Phosphate Buffered Saline
pmol	Picomole
PYR	Pyrrolidonyl Arylamidase
QLD	Queensland
RB	Rose Bengal
RFLP	Restriction Fragment Length Polymorphism
RV	Rappaport-Vassiliadis
SB	South Brisbane
SBA	Sheep Blood Agar
SEQ	South East Queensland
SG	Specific Gravity
SST	Serum Separator Tube
SXT	Trimethoprim-Sulphamethoxazole
TE	Tetracycline
TUL	Tulathromycin

V	Volts
VLM	Visceral Larva Migrans
WB	West Brisbane

CHAPTER 1

Introduction

INTRODUCTION

Established wildlife populations within peri-urban and urban environments allow the potential for dissemination of zoonotic pathogens to, from and within human-associated environments (Mackenstedt et al., 2015). Zoonotic transmission events associated with wildlife in peri-urban environments are dependent on numerous complex factors such as pathogen prevalence in host species, host species abundance, and a range of environmental and ecological factors (Bradley and Altizer, 2007). The influence of adaptation to urban environments can, as a result, have either a positive or negative influence on the ability of a pathogen to survive within these new environments. However, significant gaps in knowledge remain as a result of limited research into factors influencing transmission of zoonotic pathogens in urbanised wildlife species. The origins of human disease are, in many cases, from zoonotic pathogens harboured by wildlife (Taylor et al., 2001). More information is required to help understand the level of zoonotic pathogen carriage among wildlife living in peri-urban areas.

Within Australia, peri-urban regions are currently the location of the fastest growing human development. Furthermore, peri-urban areas are highly suited to wild canines due to the natural and modified habitats, high abundance of resources, and limited vertebrate pest control (Allen et al., 2013; Low Choy et al., 2007). Free-ranging canines, including dingoes (*Canis lupus dingo*), domestic dogs (*Canis lupus familiaris*), their cross breeds (*C. l. dingo* x *C.l. familiaris*) and foxes (*Vulpes vulpes*) are present across the majority of the Australian mainland (Fleming et al., 2014; Fleming et al., 2012b). In recent years, there has been increasing knowledge of the presence of wild dogs in peri-urban areas, particularly in south-east Queensland (SEQ) (Gentle and Allen, 2012; Low Choy et al., 2007). The frequency of wild dog sightings in residential areas is becoming progressively more common (G. Alchin, Pest Animal Management Queensland, personal communication, M. Gentle, Biosecurity Queensland, unpublished data) a phenomenon that has previously been observed with other urban vertebrate pests around the globe (Beckmann and Lackey, 2008; Gehrt, 2011). However, empirical data on important aspects of peri-urban wild dog ecology in Australia remains limited. Only recently has movement and home range data been collected (Allen et al., 2013; McNeill et al., 2016). For carnivores, such as wild dogs, home range is commonly defined by resource availability (Hayward et al., 2008). As a result, urban wildlife generally exhibit smaller home ranges than their rural counterparts (White et al., 1996). The recent data of wild dog movements suggests this remains true for peri-urban wild dogs in SEQ (McNeill et al., 2016).

Along with the suspected increased presence of these canines, reports of human conflicts with both wild dogs and foxes are also generally increasing (Gentle and Allen, 2012, Glen Alchin, Pest Animal Management Queensland, personal communication). These interactions pose direct risks to human and domestic animal safety but also suggest potential for deleterious impacts to public health. It is common for wild dogs to remain unnoticed among urban or semi-urban communities given their cryptic habits and their reduced economic impacts compared to in rural lands. As a result, reporting of economic impacts of wild dogs has been focused on rural populations (Gong et al., 2009; Hewitt, 2009; McLeod, 2004). Despite this, impacts within peri-urban zones are directly linked to public concerns regarding both human and domestic animal safety. Wild dog attacks on humans are rare but demonstrable, and dingo attacks on Fraser Island are leading to an increased awareness about the possibility and severity of such assaults (Ecosure, 2012; Lawrance and Higginbottom, 2002). Most local government agencies record a limited amount of data on dog attacks on humans, domestic animals and also wildlife, but distinguishing the difference between wild dogs and free-roaming domestic dogs is often impossible in these situations (Gentle et al., in preparation). The recent data published by Allen et al. (2013) and McNeill et al. (2016) has revealed wild dogs frequently reside within 1000 m of homes. They utilise and cross roads, visit backyards and transverse built-up areas, including nature strips, recreational ovals, and school grounds. There is also evidence that some peri-urban wild dogs may disperse large distances as young adults (Allen et al., 2013; Fleming et al., 2012a). Such movement patterns between rural and peri-urban dog populations may impact not only on public health but also livestock health, productivity and marketability of related primary products. These impacts are discussed in greater depth in Chapter 2.

The epidemiology, modes of transmission, routes of infection, parasite life cycles and pathogenicity of various infectious pathogens of canines are relatively well described (Chen et al., 2012; Deplazes et al., 2011). Wild dogs can be reservoirs of infectious agents (Mackenstedt et al., 2015) which can assist in the maintenance and/or dissemination of pathogens in other affected animal populations (Cinquepalmi et al., 2013). Infections with these pathogens in humans may result in mild or asymptomatic disease in adults and children, however some cause serious impairment to human health (Buhariwalla et al., 1996; Landmann and Prociv, 2003). These infections can result from different species of bacterial, parasitic, and viral pathogens and include vector borne diseases. Studies on domestic dogs have shown that it is more likely for a helminth infection to occur in refuge animals, as dogs attending veterinary clinics almost always have been treated with a broad spectrum anthelmintic (Palmer et al., 2008). This theory can also be applied to other pathogens such as *Campylobacter spp.* (Baker et al., 1999) and *Giardia* (Bouzid et al., 2015). Hence it is reasonable

to assume that wild dogs are more likely to be infected with pathogens than domestic dogs, and therefore contribute most towards environmental contamination with canine-associated pathogens with stages infectious to humans, other dogs, and livestock. A detailed review regarding the status of many of these zoonotic pathogens, as potentially harboured by peri-urban wild dogs, has been provided in Chapter 2. Variables associated with individual dogs or foxes such as preferential diet selection or resource availability can influence infection rates in canines. In Australia, our understanding of canine disease is largely from studies on domestic dogs and has been parasite-focused (Palmer et al., 2008; Palmer et al., 2007). With the exception of rabies (Sparkes et al., 2015) and significant pathogens such as Taeniidae family tapeworms (Jenkins, 2006; Jenkins et al., 2008; Jenkins et al., 2014b) there has been far less focus on pathogens of zoonotic and economic significance carried by wild dogs in peri-urban areas in Australia. Despite highlighting the potential impacts on human health, in depth epidemiological studies focusing on wild dog populations, particularly in peri-urban regions, are lacking. There have been only four research papers during the last ten years directly surveying wild canine pathogens in Australia (Jenkins et al., 2008; Jenkins et al., 2014b; Smout et al., 2016; Smout et al., 2013) and only two of those included urban areas (Jenkins et al., 2008; Smout et al., 2016).

The frequency of contact between wild dogs and human-associated environments is determined by the dogs' sex, age, season and habitat (Traversa, 2012). There is a need for data on pathogen prevalence, distribution, and the role of risk factors in the geographical variation of disease prevalence to assess the potential role of wild dogs in the dissemination of zoonotic pathogens to humans. A more detailed review regarding the factors influencing pathogen presence in wild dogs is presented in Chapter 2.

RESEARCH OBJECTIVES

The overall aim of the research presented in this Thesis was to conduct a broad scale investigation into the pathogens of public health and/or economic significance carried by peri-urban wild dogs across south-east Queensland and surrounding regions.

Aims:

1. To establish the current status of key zoonotic and economically significant pathogens in peri-urban wild dogs.
 - 1.1. Conduct a cross sectional survey to establish the prevalence of key pathogens in wild dogs trapped across south-east Queensland and northern New South Wales (Presented in Chapter 3).
 - 1.2. Establish quantitative burdens of *Echinococcus granulosus* (Presented in Chapter 5).

2. To investigate associations between wild dogs' diets and the presence of zoonotic and/or economically significant pathogens.
 - 2.1. To establish the occurrence and biomass of dietary items consumed by peri-urban wild dogs through analysis of whole stomach contents collected at necropsy (Presented in Chapter 4).
 - 2.2. To investigate associations between diet composition and the presence/absence of pathogens (Presented in Chapter 4).

3. To identify the most prevalent pathogens of public health and or economic significance carried by wild dogs and investigate key environmental and/or individual risk factors, including the spatial variation of pathogen presence/absence.
 - 3.1. Quantify the extent of geographical clustering for *Echinococcus granulosus* and hookworms, and describe the level of spatial dependence for point data. (Presented in Chapter 5 & 6).
 - 3.2. Investigate the role of environmental risk factors on the geographical distribution of for *Echinococcus granulosus* and hookworms, accounting for age, sex and spatial autocorrelation. (Presented in Chapter 5 & 6).
 - 3.3. Develop robust predictive prevalence maps to identify areas in SE QLD at highest risk of *E. granulosus* and hookworm infections in wild dogs (Presented in Chapter 5 & 6).

4. Conclude the significance of pathogens harboured by peri-urban wild dogs and address the implications for wild dog management.
 - 4.1. To synthesise the evidence from the four main research chapters and provide practical recommendations for future research and / or management programs (Presented in Chapter 7).

SCOPE

The research program presented in this Thesis is supported by data collected during a cross sectional survey of pathogens in peri-urban wild dogs. The survey was designed to screen for pathogens with the potential to significantly impact public health as well as to include those that have a major economic impact on the livestock industry. The selection of pathogens to be screened were based on their relevance within the study area and their significance to Australian industries. Originally a list of approximately 20 pathogens of interest was identified. Some factors (or a combination of) eliminated the possibility of testing for particular pathogens and as a result not all diseases of interest were able to be screened. In Australia and New Zealand, organisms that are

infections to humans and / or animals are classified into risk groups (1 – 4), based on: their individual pathogenicity; mode of transmission; host range; availability of preventative/treatment measures; and including the effectiveness of treatment (AS/NZS 2243.3, 2010). Risk group 1 includes organisms that are unlikely to cause human or animal disease, up to risk group four where organisms in this category present high individual and community risk, are readily transmissible, can present life threatening disease, and preventative or treatment measures are not generally available. In some cases, the AS2243.3:2010 pathogen risk classifications meant strict regulatory controls limited or practically eliminated options to detect and/or characterise in the current research. *Borrelia burgdorferi* (risk group 2) was not included due to major feasibility issues including: access to positive controls; availability of testing protocols/laboratories in Australia; and the excessive cost for overseas testing. *Coxiella burnetii* (risk group 4) was of great interest, however limitations with current testing methods for canines were apparent. Serum samples were provided to a Q-fever laboratory for future testing. Hendra Virus (risk group 4) and Australian Bat Lyssa Virus (risk group 3) were initially of interest. However, logistics and other regulatory requirements, such as access to highly contained laboratories and reagents, made testing for these unfeasible. One pathogen of interest, *Trichinella spp.* was excluded due to limited resources to test the samples and the exotic status of significant species of this pathogen in Australia (Cuttell, 2013). These have been stored and may be tested at a later date.

THESIS STRUCTURE

This Thesis consists of seven chapters: a general introductory chapter; a detailed literature review; four research chapters and a general discussion (Figure 1.1).

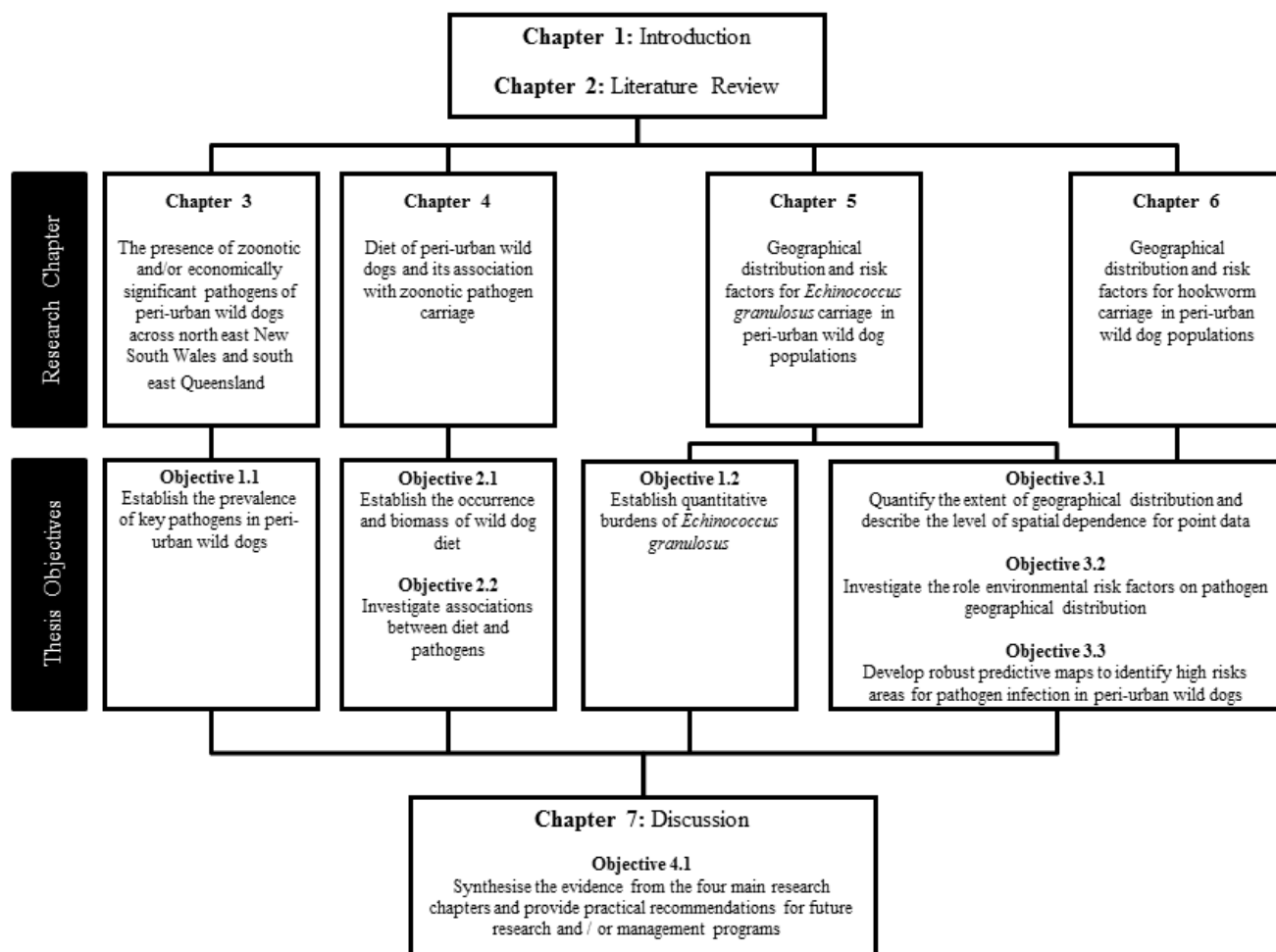


Figure 1.1 Thesis structure, based on research objectives and associated research chapters.

Chapter 1 provides a summarised background on the significance of disease in peri-urban wildlife. It also presents the scope of this Thesis explaining the reasons that some other pathogens of interest have not been addressed throughout this Thesis. It provides the rationale and objectives for the research, referring to the detailed literature review in Chapter 2 to demonstrate gaps in knowledge and highlight the current research.

In Chapter 2, a detailed literature review outlines the peri-urban wild dog situation in Australia and provides critical analysis of the current gaps in knowledge surrounding infectious diseases

harboured by peri-urban wild dogs. The review includes some history of wild dogs in Australia, the definition of peri-urban environments and wild canines, before discussing the thus far demonstrated roles wild dogs have in the maintenance of infectious pathogens within the environment. The significance this could have with the incursions of wild dogs into human associated areas is discussed.

Chapter 3 presents the first broad-scale, cross sectional survey of zoonotic pathogens harboured by peri-urban wild dogs in Australia. This foundational chapter represents an initial, exploratory exercise, required to derive deeper research questions that are further explored in the following chapters.

Chapter 4 presents the unique findings that describe the diet of peri-urban wild dogs using data collected from stomach contents. This data is utilised to assess potential associations between diet composition and the presence of zoonotic pathogens which were presented in Chapter 3. The findings from Chapter 4 lead to further investigation of risk factors pertaining specifically to *Echinococcus granulosus* which are presented in Chapter 5, and hookworms which are presented in Chapter 6.

Chapter 5 presents an in depth discussion on the risk factors associated with the presence of *Echinococcus granulosus* infection in peri-urban wild dogs. In Chapter 3, *E. granulosus* was identified as the most prevalent pathogen harboured by peri-urban wild dogs. Not only was it the most prevalent, but it has the potential to be a significant public health risk due to the nature of the parasite (e.g. environmentally resistant eggs) and the presentation of disease in humans (hydatid disease). After the discovery that dietary selection may influence the presence of *E. granulosus* in Chapter 4, further investigation was conducted into the role that environmental factors may have in the presence of the pathogen. This chapter presents those findings.

Chapter 6 presents an in depth discussion on the risk factors associated with the presence of hookworm infections in peri-urban wild dogs. In Chapter 4, hookworms were identified as the second most prevalent zoonotic pathogen harboured amongst the study population. The study location, south-east Queensland, theoretically provides a suitable environment for hookworm survival. Hence, this chapter aims to identify and quantify which climatic environmental factors (if any) were influencing the presence/absence of the parasite in the study area.

Chapter 7 presents the general discussion aiming to summarise the main findings across the research chapters. Here, the study limitations, knowledge gaps that remain, and suggestions for future research are addressed. Practical implications and recommendations are provided for wild dog management in relation to control of the zoonotic pathogens that they carry.

The findings of the research studies presented in this Thesis provide new and updated data on the pathogen prevalence in peri-urban wild dogs. Importantly, it provides an epidemiological approach where the pathogen status and the ecological aspects of peri-urban wild dogs are considered. As a result it presents a sophisticated and refreshing approach in describing the epidemiology of zoonotic pathogens in wild dogs in peri urban areas in SEQ.

CHAPTER 2

Literature review

Peri-urban wild dogs as a potential source of zoonotic
pathogens to human communities in Australia

WILD DOGS IN AUSTRALIA

Dingoes (*Canis lupus dingo*) are decedents of wolves, their ancestry tracking back through mainland south-east Asia (Oskarsson et al., 2012). Introduction of dingoes to the Australian mainland occurred approximately 4000 years ago. Some Australians consider them to be a native animal because they were present on the continent when European settlers arrived in the 18th century (Fleming et al., 2001). However, the arrival of European settlers also included the introduction of domestic dogs (*Canis familiaris*). Dingoes and domestic dogs have since interbred, resulting in a large and now common population of hybrid animals (Corbett, 2001a). As a result, dingoes, feral domestic dogs, and their cross breeds are often collectively regarded as 'wild dogs'. The introduction of domestic dog genetics into dingo populations has occurred across the Australian mainland, however is more prominent amongst populations of wild dogs located closer to the densely human populated east coast (Stephens et al., 2015).

Recent data suggests that 90% of wild dogs in south-east Queensland possess a combination of dingo and domestic dog genetics (Gentle et al, unpublished data), with no area in Australia free of hybridisation (Stephens et al., 2015). This has caused an increasingly difficult task in balancing conservation of the dingo and control of wild dogs around farming land (Elledge et al., 2008). Legislation regarding conservation of dingoes differs according to State legislation where generally they are protected as a native species in areas such as national parks, but also declared as a pest, allowing control on private land (Invasive Animals CRC, 2016). In Queensland, the dingo is protected in national parks under the *Nature and Conservation Act* 1992, however, dingoes and wild dogs are declared a pest under the *Rural Lands Protection Act* 1985. In the field, it can be difficult to distinguish the difference between dingoes and hybrid wild dogs. Generally, dingos exhibit erect ears, a bushy tail, and ginger/tan, black and tan, black or white coloured coats. Some dingoes will feature white paws and/or have a white tip on their tail, however, the amount of white can be variable and is not a reliable defining feature (Elledge et al., 2008). The coat colour of hybrid animals may vary to include brindle or be patchy ginger and white, patchy black and white, and can vary geographically (Radford et al., 2012). Hence, it still remains difficult and often impossible to differentiate between pure dingo and hybrid animals due to the lack of knowledge about the relationship between the phenotype and the genotype of coat colour (Fleming et al., 2001; Purcell, 2010). There are other methods of estimating the extent of hybridisation. These typically involve either assessing the skull morphology of the dog or DNA testing, but these methods are limited in their ability to distinguish hybrid wild dogs and/or are not practical in a field situation (Elledge et al., 2008). For this review, all dogs will be classified as wild dogs, taking into account that this classification may include both dingoes and hybrid animals.

DISTRIBUTION AND ABUNDANCE

Wild dogs inhabit the majority of mainland Australia, covering 6.3 million square kilometres (82.8%). This includes Tasmania where wild dogs have been recently recorded (West, 2008) (Figure 2.1). Before European settlement, dingoes were assumed to be present across the entire mainland, however, with an increase in farming activities, wild dogs were eliminated from approximately 25 percent of Australia to accommodate sheep (*Ovis aries*) production (Fleming et al., 2001). This was assisted through the erection of an exclusion fence covering a total of 5164 km across South Australia, New South Wales and Queensland (Downward and Bromell, 1990). Although the presence of the fence limits free passage of dogs, wild dogs regularly pass through into the southern parts of Australia through gaps made by other species such as wombats. Currently there is a lack of knowledge about the abundance of wild dogs in any specific area of Australia. Part of this is likely related to the fact that there is no standard measure of wild dog population. Studies generally assess wild dog populations through passive activity indices including tracking stations (plots), or active tracking stations including baiting stations. Many of the studies previously conducted have come to conclusions based on results that may have been confounded by season, habitat or methodological inconsistencies (Allen et al., 2011). Radio tracking and mark and recapture studies have been conducted although the data is relatively old. Mcilroy et al. (1986) found wild dog densities of 0.178 dog per square kilometre in the Kosciusko national park, NSW and Fleming et al. (2001) suggests densities range from 0.1 to 0.3 dogs per square kilometre in north eastern NSW, although, this data dates back at least 17 years.

GENERAL ECOLOGY

Commonly, wild dogs live in groups with a dominant pair, subordinate animals, which may or may not include their offspring, and juveniles. Pure dingoes breed only once a year, with the female becoming receptive only once during the breeding season from March to May (autumn) (Catling et al., 1992). However, a reported increase in young pups within the population at all times of the year suggests that hybrid animals may not necessarily adhere to the general timeline (Jones and Stevens, 1988). Domestic bitches can breed up to two or three times a year, with males fertile year round (Gavrilovic et al., 2008). It would be an unlikely occurrence for wild dogs to breed more than once a year due to protein requirements of pregnancy and lactation, however it has been suggested that the influence of domestic dog genetics within the wild population has led to the change in breeding seasonality (Catling et al., 1992). However, hybrid species generally remain more genetically similar to dingoes than domestic dogs (Gentle et al, unpublished data) and recent research suggests that dingo-dog hybrids exhibit similar breeding seasonality to dingoes (Cursino et al., 2017). So, assuming wild dogs follow the typical breeding season, pups are born during the winter months

(June to August). The presence of pups outside of the typical breeding season is therefore most likely associated with subsequent oestrous cycles, where a late pregnancy occurs, because for unknown reasons, the first one had failed. Between the ages of six to twelve months pups become independent and begin to disperse, although some may remain as subordinate animals within their family pack (Corbett, 2001b). The ability for wild dogs to disperse relies on natural deaths within the overall population but is also influenced by humans through the intensity of control in certain areas.

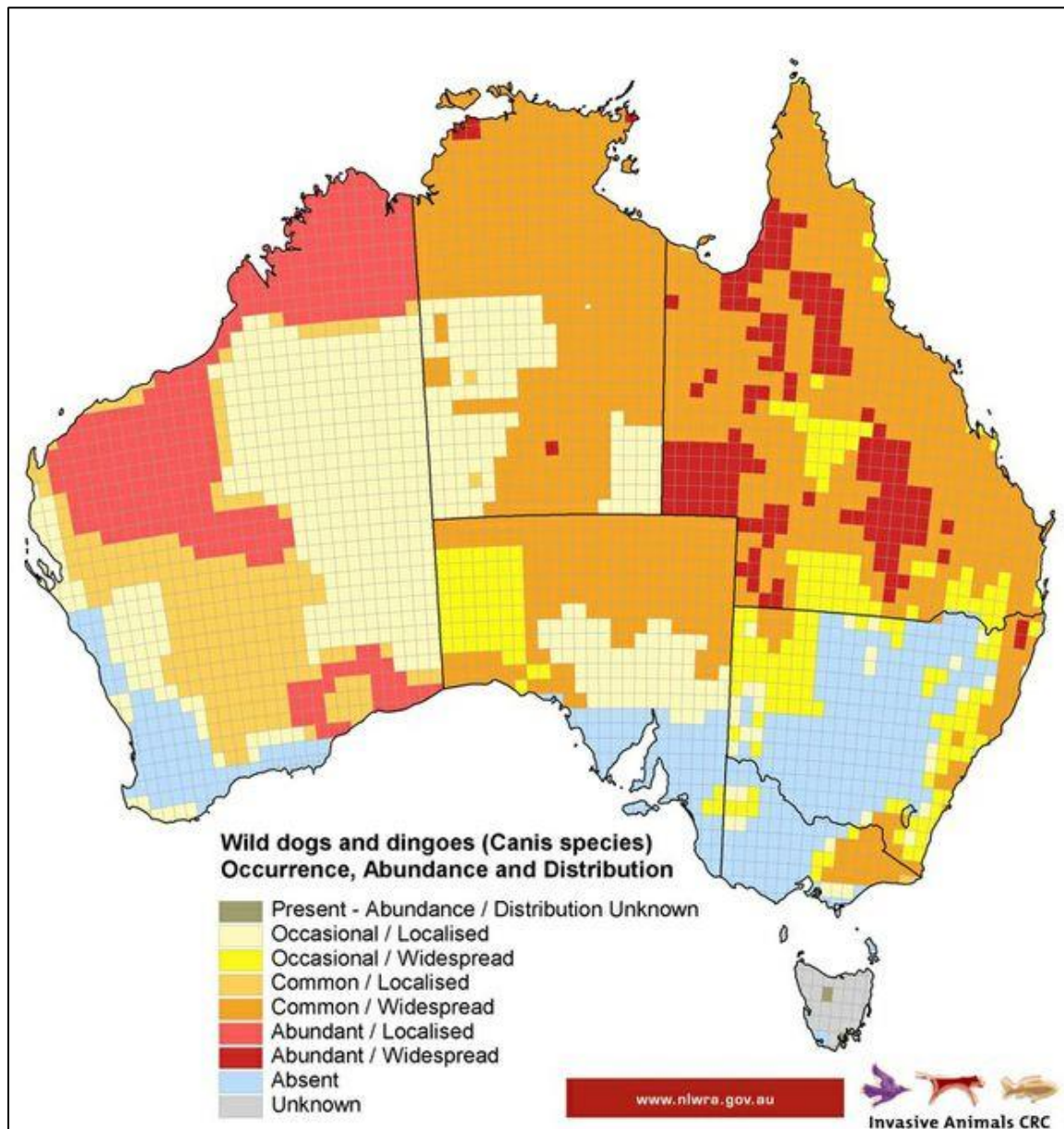


Figure 2.1 Wild dog distribution occurrence, abundance and distribution in Australia, 2007. Image from www.pestsmart.org.au

DIET

Wild dog diets in Australia have been extensively studied through the detection of prey species in scats. It is well understood that wild dogs are generalist predators, with the ability to consume a large range of different dietary items including but not limited to, large, medium and small mammal species, birds, reptiles, insects, fruits and other plant materials (Allen et al., 2016; Claridge et al., 2010; Corbett, 2001b; Cupples et al., 2011; Whitehouse, 1977). They have also been known to take advantage of human provided food sources (Newsome et al., 2013a; Newsome et al., 2014). However, in urban areas, where there is generally an abundance of anthropogenic food resources year round, it is unknown if wild dogs frequently take advantage of this. In Zurich, foxes within the urban environment were found to frequently consume anthropogenic sources of food, and similar results have been reported in London, Oxford, and Bristol (Contesse et al., 2004; Hegglin et al., 2007). Likewise, in the Chicago metropolitan area, coyotes were found to consume human associated food items if they ventured into developed areas of the city (Murray et al., 2015). Hence, it would be likely that Australian wild dogs would opportunistically consume anthropogenic food resources if they become available to them. Recent dietary analyses of Australian peri-urban wild dogs suggest that human sourced food items are not of significance as mammalian prey species were predominantly detected within scats (Allen et al., 2016). However, as mentioned in Allen et al. (2016), scat studies may not be sensitive in detecting anthropogenic food items, often because of their increased digestibility. Diet composition depends heavily on the availability and abundance of prey, although some foods may be preferentially consumed (Corbett and Newsome, 1987). If the diet of urban and peri-urban wild dogs differs greatly from those of rural wild dogs it could be likely that there will be variance in the pathogens that they carry.

DEFINING PERI-URBAN

The term ‘peri-urban’ is loosely defined, but in general terms refers to areas in a ‘transitional zone’ adjacent to, and influenced by, urban centres (Low Choy et al., 2007). They are neither classified as urban nor rural and are comprised of a fragmented mixture of rural, residential and commercial land uses. The diversity and dynamic nature of land use makes it difficult to determine both the inner and outer boundaries of the peri-urban zones. There is often a combination of subdivision, fragmentation and land use conversions, resulting in a temporary mix of rural and urban activities and functions (Butt, 2013). As the Australian population increases, the popularity of peri-urban living is also growing, with cultural and lifestyle choices being major attractants to the areas (Low Choy et al., 2008).

WILD DOGS IN PERI-URBAN REGIONS

Understanding peri-urban wild dog ecology is important to mitigate not only wildlife-human conflicts, but to assist with improved and adaptive management programs through identifying key ecological requirements of the predators. Wild dogs in peri-urban regions utilise much smaller home ranges than their rural counterparts (Allen et al., 2013; McNeill et al., 2016). This is a phenomenon that is common to other urban carnivore species (Bateman and Fleming, 2012; White et al., 1996), and is often associated with high resource abundance (Corbett, 2001b; Newsome et al., 2013b). Peri-urban and urban wild dogs are flexible with their spatial requirements, residing in high risk urban locations, but reducing movement patterns during times of high human activity (McNeill et al., 2016). Recent GPS tracking data has revealed that wild dogs frequently utilise and traverse areas such as recreational ovals (including school grounds), parkland, areas of native vegetation, nature strips and other built up areas (Allen et al., 2013). Throughout the tracking period of peri-urban wild dogs, they were found to be within 700 m (Allen et al., 2013) or 1000 m (McNeill et al., 2016) of houses and buildings at all times. In relation to wild dog management there are a number of significant issues associated with this, which will be discussed further in following chapters.

ECONOMIC IMPLICATIONS OF WILD DOGS

Despite the availability of reports discussing the economic impacts of vertebrate pest species, such reports are few and each has stated values that are inconsistent with each other. In Queensland (one of eight states and territories in Australia, comprising 22.5% of land area), wild dogs are estimated to cause a total economic loss of around \$67 million dollars annually (Hewitt, 2009). This is considerably higher than the \$66.3 million national estimate by McLeod (2004), and the \$48.5 million national estimated by Gong et al. (2009). Gong et al. (2009) only considers the impacts of wild dogs and not the management and research costs, which were discussed at a commonwealth and state level, but not specifically for wild dogs. The large differences between these numbers shows that evaluating economic impact of wild dogs is not a simple task, and that optimal, standardised methods are yet to be developed (Fleming et al., 2012a). The cost of wild dogs can be separated into distinct categories, discussed below.

Management, fencing and research costs

In the report by McLeod (2004), the estimated management, fencing and research costs totalled \$18 million dollars for the entire country. McLeod (2004) utilised information published in a report from Bomford and Hart (2002). They estimated landholder spending costs based on a prediction of an average spend of \$250 per farm per year for all feral animal control. Government research costs were based on estimates where records were not available and did not include salaries or costs for

any associated infrastructure, such as the wild dog barrier fence (Bomfort and Hart, 2002). The annual operating budget for the wild dog barrier fence alone was \$1.9 million dollars between 2009-2010, with that cost shared 50:50 between state and local governments (DEEDI, 2011). A more recent report focusing on the effects of wild dogs on the Queensland grazing industry estimated costs just short of \$20 million dollars annually (Hewitt, 2009). Hewitt (2009) based his estimates through surveying producers of cattle, sheep, goats and mixed farms. It was determined that: cattle producers spend \$11,460,498 annually on wild dog control methods; that sheep and/or goat producers spend \$2,248,642; local governments contribute \$2,623,543; \$1,870,316 goes towards maintaining the wild dog barrier fence; and the state government adds \$1,754,000 (Hewitt, 2009). Gong et al. (2009) found that for the year 2007-2008 the commonwealth government spent at least \$12.638 million dollars on pest control and research, with the state governments all spending different amounts that varied with specific pest control issues. Queensland was predicted to spend \$9.709 million dollars for the same time frame, but they did not mention how much was as a result of wild dog management.

Production loss through predation

Sheep production in Queensland has been gradually decreasing over the past 10 years. There are a number of possible explanations for the decline including: drought, decreased wool prices, and difficulty in employing staff. However, according to several graziers, predation by pests, mostly wild dogs, has been the 'tip of the iceberg' in their decision to leave the sheep production industry (RuralManagementPartners, 2004). Although there is no conclusive data as to the amount of sheep that are preyed upon by wild dogs each year, studies have shown that the nature of sheep makes them a relatively easy prey item and many attacks can be made by one dog, in excess of its nutritional requirements (Allen and Fleming, 2004). Based on survey results of 105 sheep and goat producers in Queensland, wild dog attacks are estimated to cost producers on average \$3.87 per sheep per year, equating to a total annual loss of \$16,950,000 (Hewitt, 2009). Estimates based on annual economic surplus losses by wild dogs were predicted to cost the national beef industry \$26.68 million (Gong et al., 2009). However, when cattle farmers in Queensland were asked to estimate the amount lost due to wild dog attacks on calves, it was predicted that \$22,840,000 is lost annually plus approximately another \$2,068,000 lost at either the saleyards or at processing due to dog inflicted bites (Hewitt, 2009).

Production loss through disease

Wild dogs have the potential to carry infection agents that result in significant losses of production. Overlap in ecological regions allows for wild dogs to spread pathogens into the environment where

grazing species become infected. Losses from disease could be related to impacts on animal growth such as reduced animal appetite and weight loss, it could be related to implications of reproductive output, or include losses at the abattoir due to carcass condemnation or trimming of meat from cysts in the tissues.

Neospora caninum

Neospora caninum is an obligate intracellular, coccidian, protozoan parasite. It causes neosporosis in cattle, a leading cause of bovine reproductive failure (Dubey, 2003). There are three infective stages in the lifecycle of *N. caninum*: tachyzoites, bradyzoites (tissue cysts) and sporozoites (within the oocytes) (Dubey et al., 2007). The first two, tachyzoites and bradyzoites, are found intracellularly within tissues of intermediate hosts (Dubey, 2003). Oocytes are found only in the faeces of a definitive host, but there is no data regarding the survival time of oocytes in the environment (Dubey et al., 2006; Dubey et al., 2007). Canines become infected through eating bradyzoites in the tissues of an intermediate host, typically a herbivore. Once the canine is infected it excretes the oocytes in its faeces which contaminate the environment, including water bodies. Intermediate hosts then ingest the oocytes, where the sporozoites invade the wall of the intestinal tract and develop into tachyzoites (Goodswen et al., 2013). Once the intermediate host is infected it can vertically transmit disease to its progeny and it remains infected for life.

In cattle, the most common clinical manifestation of the disease is abortion, which may occur from any time between three months to full term gestation (Dubey et al., 2006). Calves that are born commonly present as clinically normal, despite being congenitally infected (Williams et al., 2009) although some calves may present neurological signs expressed through limb dysfunction (Dubey and Lindsay, 1996). There is the rare opportunity also that calves can be born without the disease (Guy et al., 2001).

Globally, the prevalence of *N. caninum* is quite high. In Queensland, beef cattle have a suggested mean state wide seroprevalence of 13.8%, with south east Queensland having the highest prevalence of 20.9% (Taylor and Landmann, 2003). Canines, including domestic dogs, dingoes, grey wolves (*Canis lupus*) and coyotes (*Canis latrans*) are the definitive hosts of *N. caninum* (Dubey et al., 2011; Gondim et al., 2004; King et al., 2010; McAllister et al., 1998). However, they can also be intermediate hosts, meaning not only they shed the oocytes in their faeces but also harbour tachyzoites and bradyzoites. Hence, dogs may transmit the parasite through both horizontal and vertical transmission. Any breed, sex or age can be infected, although the most severe cases are generally reported in young dogs that have been congenitally infected (Dubey, 1992; Dubey, 2003). A wide variety of studies have shown that neosporosis can cause a vast range of clinical signs in

dogs (Barber and Trees, 1996; Garosi et al., 2010; Hay et al., 1990; Odin and Dubey, 1993; Patitucci et al., 1997). However, the most obvious signs of infection in dogs include the development of either ataxia or paresis, more commonly in their hind limbs, which can result in varying severities of paralysis with the pelvic limbs often in rigid hyperextension (Barber and Trees, 1996; Mayhew et al., 1991). The severity of neurological effects depends on the site of bradyzoite invasion (Lorenzo et al., 2002).

Dingoes have been found to be definitive hosts of *Neospora*, although this was through experimental infection (King et al., 2010). There is no evidence yet to suggest that wild dogs naturally shed oocytes, however, dogs in NSW and QLD have been found to be seropositive to *N. caninum* (Barber et al., 1997). It is possible that a major source of infection for wild dogs is through the consumption of infected placental material of cattle. If this is the case, it has significant implications to transmission risk as dogs have been shown to produce a significantly greater number of oocytes though this exposure in comparison to becoming infected through consumption of an intermediate host, such as mice (Gondim et al., 2002). If wild dogs are infected through consumption of placental material then a single infected dog is capable of infecting numerous intermediate hosts. Although there is a lack of scientific evidence supporting this hypothesis, it is likely that wild dogs contribute to the maintenance of neosporosis in cattle in Australia. Wild dogs have been estimated to cause \$3.14 million worth of damage per year due to transmission of *N. caninum* (Hewitt, 2009). However, King et al. (2011) suggest that farm dogs are more efficient vectors for *N. caninum* and hence are a more likely source of horizontal transmission to livestock than wild dogs. More information is required to determine the roles of domestic dogs and wild dogs in their respective contributions to transmission of *N. caninum* to cattle. If domestic dogs were the main vector, then management of neosporosis within cattle populations could be simplified, as control may be managed through responsible ownership of farm dogs and minimising their access to intermediate stages of the parasite. But, despite the lack of shedding evidence amongst the wild dog population, it is possible for a single infected dog to excrete in excess of 500,000 oocytes (Gondim et al., 2005). Hence, if infection prevalence amongst the wild dog population is low, reducing the numbers of wild dogs may not result in a reduction of the transmission rate at the property level (Gondim et al., 2002).

Taenia spp.

The family Taeniidae are cestode tapeworms that comprise approximately 43 species and three subspecies (Hoberg, 2006). In Australia, there are several species that infect canines, however, of these there are only two species of concern to livestock: *T. ovis* and *T. hydatigena*. Canines are definitive

hosts, with livestock becoming infected through ingestion of infective eggs that have been passed onto the pasture from the faeces of infected dogs. Sheep infected with *T. ovis* and/or *T. hydatigena* are reported to be widespread and common within slaughterhouses across mainland Australia (Jenkins et al., 2014b). *T. ovis* is commonly referred to as sheep measles due to the presence of small cysts that develop in the muscles. Cysts remain viable in sheep for approximately 1-2 months before mineralising into hard calcified nodules (Ransom, 1913). This can cause significant financial loss for the Australian meat industry through carcass condemnation at the abattoir or downgrading of meat (Jenkins et al., 2014b). The financial impact of *T. hydatigena* is less important but larval stages of *T. hydatigena* may cause tissue damage leading to condemnation of infected organs (Jenkins et al., 2014b).

Domestic dogs are considered to be the main definitive host of both *T. ovis* and *T. hydatigena* in Australia (Dybing et al., 2013). In 1972 the prevalence of *T. ovis* and *T. hydatigena* in 204 Victorian dingoes and feral dogs were 0% and 7.5%, respectively (Coman, 1972). A more recent survey of over 1400 rural domestic farm dogs also failed to identify *T. ovis* in a single dog (Jenkins et al., 2014a). The low incidence rates of both *T. ovis* and *T. hydatigena* in comparison to other taeniid cestodes was thought to be related to the minimal presence of livestock species in their stomach contents, suggesting they were not a staple dietary item, possibly because of other abundant medium sized prey such as wallabies (Coman and Ryan, 1974). However, research has suggested that perhaps foxes may be a more suitable host for *T. ovis* than wild dogs (Jenkins et al., 2014b; Pullar, 1946) although a number of reports dispute this claim (Coman and Ryan, 1974; Dybing et al., 2013). Hence, the situation regarding the role of wild canines as definitive hosts for *T. ovis* and *T. hydatigena* remains controversial. The most recent and comprehensive research concluded that foxes, not wild dogs, are the most significant definitive host for *T. ovis*, whereas wild dogs represent a more significant host for *T. hydatigena* (Jenkins et al., 2014b). Humans can be infected with *Taenia spp.* However, the lifecycles for these zoonotic species do not involve canines (Hoberg, 2006).

POTENTIAL PUBLIC HEALTH RISKS ASSOCIATED WITH WILD DOGS

Wild dogs can be reservoirs of infectious agents (Mackenstedt et al., 2015) and can assist in the maintenance and/or dissemination of pathogens in other affected animal populations (Cinquelpalmi et al., 2013). Infections may result in mild or asymptomatic disease in adults and children, however some cause serious impairment to human health (Landmann & Prociv, 2003, Buhariwalla et al., 1996, Avashia et al., 2004). These infections can result from different species of bacterial, parasitic, and viral pathogens and include vector borne diseases. Studies on domestic dogs have shown that it

is more likely for a helminth infection to occur in refuge animals, as dogs attending veterinary clinics almost always have been treated with a broad spectrum anthelmintic (Palmer et al., 2008).

The ability or potential for a wild dog to become infected with a pathogen can depend on numerous factors (Figure 2.2). Knowledge of the potential ways that canines become infected can assist in developing effective strategies to manage infectious pathogens in conjunction with routine wild dog management. Understanding how dogs become infected and what external factors might be influencing the presence (or absence) of disease can assist pest managers with the knowledge they need to manage disease risk through management of dog populations.

Below, we discuss potential pathogens of public health significance that can infect wild dogs, detailing the current knowledge surrounding infection in both humans and wild dogs.

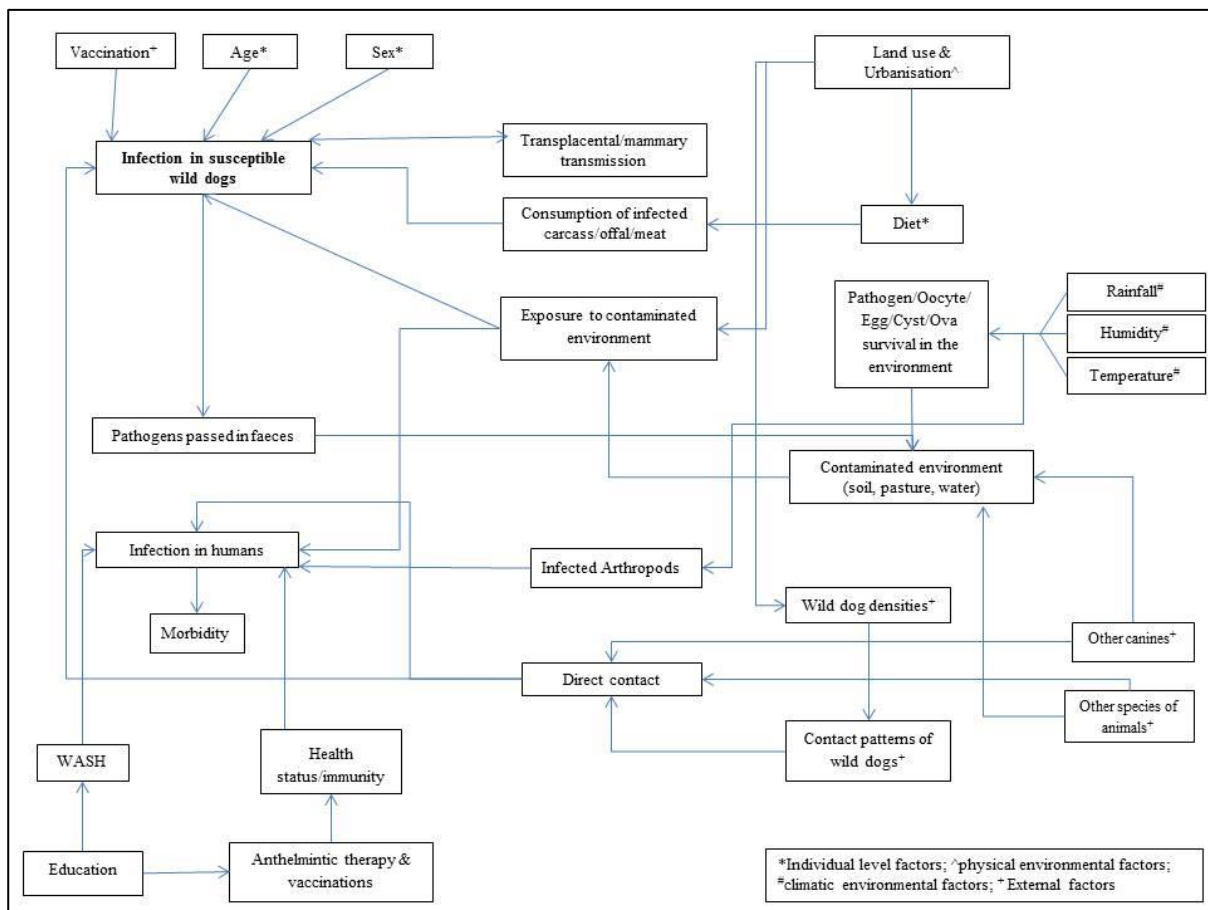


Figure 2.2 Transmission pathway diagram showcasing potential influencing factors (individual level, physical environmental, climatic and external) of general pathogen infection in wild dogs and humans.

Brucella suis

Brucellosis is a common and widespread bacterial disease throughout the world (Pappas et al., 2006). It is readily transmitted between wildlife, domestic animals and humans with traditional risk factors for human infection being exposure to infected livestock and their products. In Australia, *B. suis* and *B. ovis* are the only species present after the eradication of *B. abortus* in 1989 (Turner, 2011). Of these, *B. suis* is the only species that causes disease in humans. Currently, there are five different biovars of *B. suis* with only biovars 1 and 3 capable of producing significant disease in humans, and only biovar 1 thought to be present within Australia. Humans become infected with *B. suis* when: open skin wounds come into contact with an infected animal's bodily fluids such as blood, saliva, or urine; when infected meat or dairy products are consumed; or through inhalation or mucosal exposure to aerosolised bacteria (Young, 1995). Infection in previously healthy humans is not generally lethal, however, it can result in chronic disease showing clinical signs ranging from fever and headaches to endocarditis, meningoencephalitis, arthritis, orchitis and psychological disturbance. Australia is known to have a low infection rate, with a reported 0.2 cases per 100,000 people (Newman et al., 2008). Since 1991, 83% of cases have been reported from Queensland, and most are positively correlated with exposure to feral pigs, through recreational or occupational hunting (Irwin et al., 2009; Massey et al., 2011; Milton et al., 2010). There is limited data about the prevalence of *B. suis* in pigs in Australia. Research suggested that *B. suis* infection in feral pigs was limited to Queensland (Mason and Fleming, 1999). However, recently it was detected in feral pigs from NSW with an overall seroprevalence of 2.9% (Ridoutt et al., 2014). There has not been any recorded detection of *B. suis* among the Australian domestic pig population.

Feral pig hunting is often aided by the use of hunting dogs (Sparkes et al., 2016a). Recently, trends in canine *B. suis* infection have been documented in association with feral pig hunting (Mor et al., 2016; Ramamoorthy et al., 2011). Hunting dogs are likely to become infected through blood borne contact with infected swine, although ingestion of offal, tissue or foetal material is also a possible cause of infection. Between 2011 and 2015, there has been a 17 fold increase in the reporting of hunting dogs that have been diagnosed with *B. suis* biovar 1 infection in NSW (Mor et al., 2016). Pig hunting and pet dogs have also been incidentally diagnosed with *B. suis* infection in SE Queensland (R. Cobbold, personal communication). Currently, the prevalence of this pathogen in wild dogs is unknown. However, feral pigs and wild dogs are known to co-habit landscapes across Australia. Additionally, dietary studies have shown that wild dogs consume feral pigs at low levels (Allen et al., 2016). Hence, it is reasonable to suggest that the population of wild dogs may also be infected. The risk to humans residing in a household with an infected dog remains unknown (Woldemeskel, 2013), although plausible according to Mor et al. (2016). Similarly, the public

health impact from an infected wild dog in peri-urban regions is unknown. Despite this, the recent increased trend in testing and positive identification of the pathogen is leading to new investigations of the role of dogs (both hunting and wild) in the epidemiology of *B. suis* in Australia.

Campylobacter spp.

Campylobacter is the most common bacterial cause of human gastroenteritis throughout the world (Wilson et al., 2008). The most common species that cause human infection are *C. jejuni* followed by *C. coli* (Gillespie et al., 2002; Whiley et al., 2013). This is the most notified enteric disease in Australia (NNDSS, 2013). There were 16,966 cases reported in 2010, which accounts for 112 cases per 100,000 population (Milton et al., 2010). Four thousand seven hundred and eighty eight of these cases were reported from Queensland with only Victoria having a higher number of infections. This is much higher than the occurrence rates reported for the USA in 2009, where there were 13.02 cases per 100,000 population (CDC, 2009). Causes for the high rates in Australia are unknown, but could be related to a number of factors including environmental exposures, reporting rates and food consumption patterns (Unicomb et al., 2008). The most common symptoms in humans include diarrhoea and abdominal pain, with vomiting uncommon (Moore et al., 2005). In rare cases, likely when the immune system has previously been compromised, more serious signs of disease can develop. The most significant of these is Guillain-Barré syndrome (McCarthy and Giesecke, 2001; Nachamkin, 2001).

Campylobacter spp. are mostly considered foodborne pathogens, particularly through contaminated food of poultry origin (Hall et al., 2005; Stafford et al., 2008). However, environmental sources, including soil and water, and animal contact have an important contribution to human infection (Pintar et al., 2015; Whiley et al., 2013). Domestic dogs have been identified as a risk factor for human infection (Tenkate & Stafford, 2001, Stafford et al., 2007) with dogs less than one year old being the most susceptible to infection (Hald et al., 2004; Sandberg et al., 2002; Westgarth et al., 2009). Published information on the prevalence of *Campylobacter spp.* in canines in Australia dates back to 1999. Baker et al. (1999) found that 34% of south Australian stray and owned dogs were infected with *C. upsaliensis*, 7% were infected with *C. jejuni* and 2% with *C. coli*. Only one study has opportunistically identified the pathogen in wild dog faeces (Allen, 2006) but, 43.8% of shelter dogs were identified as thermophilic *Campylobacter* positive in 2007-2008 (Thi Thu Hong, 2010). It is difficult to suggest the extent that wild dogs contribute to environmental contamination given the pathogen has such a wide host range, including wildlife (Mohan, 2015) and livestock species (Ogden et al., 2009).

Coxiella burnetii

Coxiella burnetii, the aetiological agent of Q-fever, can be transmitted through inhalation of aerosols of bodily fluids, but also by contact with infected animal hair, hides, wool or also fomites such as straw (Marrie, 1990). It is common across the globe, with the exception of New Zealand. Clinical signs in humans are present in approximately 50% of cases. These may include common flu like symptoms such as fever, chills or sweats, fatigue, headache and muscle pain (Spelman, 1982). More severe cases could include digestive pain, chest pain and / or cough with the potential to develop pneumonia or hepatitis. A rare percentage of people may develop chronic Q-fever which, if left untreated, can be a very serious and life threatening disease. Prevention is possible through the administration of a vaccination to humans (Sellens et al., 2016).

In Australia, there have been approximately 1.7 human cases per 100,000 population reported each year from 2008 to 2012 (NNDSS, 2015). Queensland, particularly the northern regions, frequently exceeds the national average with 4.2 cases per 100,000 population in 2012 alone. Most human infections occur through airborne transmission from animal reservoirs. People at highest risk are those who have had frequent contact with cattle, sheep or goats, particularly contact with fluids and materials during parturition in these ruminants (McQuiston and Childs, 2002). It has been suggested that domestic pets may be a possible source of Q-fever for humans that were considered to be traditionally low risk populations (Cooper et al., 2011; Marrie et al., 1988). In Townsville, apparently healthy domestic dogs attending veterinary clinics were tested for the presence of anti-*C. burnetii* phase I or II antibodies (Cooper et al., 2011). Twenty one point eight percent of dogs tested seropositive with the most common risk factor shown to be contact with wildlife. Domestic, shelter and breeding dog populations in Sydney have also been found to be 3%, 1.9%, and 2.3%, respectively, seropositive to the bacterium, with Aboriginal community dogs 2.8 times more likely to be infected than those populations (Shapiro et al., 2016). A human outbreak of Q fever has been reported after contact with a parturient bitch (Buhariwalla et al., 1996), however, there is limited research surrounding this topic. Wild dogs in Northern Queensland have been shown to be seropositive to *C. burnetii* (Cooper et al., 2012). Considering the contact rates between wild dogs and potentially Q fever infected wildlife such as flying foxes and koalas (Tozer et al., 2014), kangaroos (Banazis et al., 2010), brush tail possums (Cooper et al., 2012) and bandicoots (Bennett et al., 2011; Cooper et al., 2012) are likely to be frequent, there is a strong potential for *C. burnetii* to be present in the peri-urban wild dog population. This may provide an additional source of infection for domestic dogs, and resultant links to human infection, but also provide a direct link between peri-urban wild dogs and human disease. However further research in canines to identify

active antigen within excreta is necessary before decisions regarding public health risk can be made (Shapiro et al., 2016).

Echinococcus granulosus

E. granulosus, a highly significant zoonotic pathogen, is present in its intermediate phase as hydatid cysts in the internal organs of intermediate hosts such as livestock and macropods (Jenkins, 2006). Transmission occurs through a predator-prey relationship, with dog-sheep, and/or dog-macropods comprising the primary domestic and sylvatic cycles, respectively, in Australia, with opportunity for overlap. Wild canines consume the cysts of infected intermediate hosts, and as definitive hosts of the tapeworm, shed eggs into the environment which become infective to intermediate hosts, and the cycle continues (Jenkins & MacPherson, 2003). Humans are accidental hosts and become infected through consumption of eggs sourced from the environment or through contact with a definitive host (Moro & Schantz, 2009, Torgerson & Budke, 2003). Once ingested, oncospheres penetrate the intestinal mucosa and enter into the blood stream. The oncospheres attach to internal organs, commonly the liver or lungs, but occasionally muscle, kidneys, eyes and brain where they develop into cysts. Cysts expand, causing pressure on the vital organs or damaging the surrounding tissues, which may or may not result in clinical signs of the disease. In humans this is classified as cystic echinococcosis (CE) whilst the disease is generally referred to as hydatid disease. Hydatid disease has not been a notifiable disease in Australia since 1996 (2008 in Queensland), however from 1987-1992 only 17 cases of 321 patients treated for hydatid disease were notified (Jenkins & Power, 1996). This not only suggests human infection with hydatid disease is greatly underestimated, even when notifiable, but also that the true potential impacts on public health are not understood nor recognised.

Research on *E. granulosus* in Australia has focused on the prevalence of tapeworms in rural farm dogs, wild dogs and hydatid-infected intermediate hosts including macropods. In wild dogs, the prevalence of tapeworms can reach as high as 93% in Victoria (Grainger & Jenkins, 1996) and between 25% and 100% in New South Wales (Jenkins & Morris, 2003). This is not surprising given rural-living wild dogs frequently interact with livestock species and macropods. More recently, studies have expanded to include peri-urban/urban animals, but current knowledge is limited to two small scale studies in eastern Queensland. These studies revealed that 46.3% of wild dogs were infected in the Maroochy shire (Jenkins et al., 2008) and six of 20 (30%) wild dogs were infected in Townsville (Brown & Copeman, 2003). Despite the high level of infection in wild dogs and the recognition of the potential public health impacts, detailed epidemiological studies are lacking within these regions.

Escherichia coli

Escherichia coli is a versatile and widespread bacterial pathogen, found most commonly living in symbiosis in intestinal tracts and the faeces of most vertebrates (Tenaillon et al., 2010). The bacteria comprises both commensal and pathogenic strains (Leimbach et al., 2013). Infection by pathogenic strains in humans is often from direct contact with animals or from consumption of contaminated water or food products (Ferens and Hovde, 2011). Human infection is often sporadic and usually presents as generic symptoms of gastrointestinal pain, including diarrhoea (often bloody) and abdominal cramps, with the potential for further complications to develop (Combs et al., 2005). The reported incidence of human infection with pathogenic *E. coli* is less common than some of the other enteric bacterial pathogens such as *Campylobacter spp.*. However, because of the potential for severe complications to occur, including extra-intestinal symptoms associated with some pathotypes such as enterohaemorrhagic *E. coli*, it remains a significant public health concern (Pennington, 2010). The species and / or strains of bacterial pathogens present within wild dog populations have not previously been investigated. Hence, the role of wild dogs in transmission of pathogenic *E. coli* is currently unknown.

Antimicrobial resistance (AMR) of bacterial pathogens is a serious and growing concern within animal and human health sectors (Silbergeld et al., 2008). The development of AMR within livestock has the ability to influence resistance genes in human populations through the food chain (Obeng et al., 2013, Hart et al., 2004). The domestic animal population is also known to contribute to the zoonotic exchange of AMR (Platell et al., 2011, Johnson and Clabots, 2006). Within the scope of this thesis, *E. coli* has been screened due to its potential as a zoonotic pathogen but more importantly, as an indicator for Gram negative AMR pathogens. The presence of AMR *E. coli* within the peri-urban wild dog population could indicate another potential pathway for the zoonotic transmission of AMR pathogens or genes. A detailed review surrounding AMR is beyond the scope of this thesis, however, readers can be referred to several previously published reviews (Koch et al., 2017, Pomba et al., 2017, Cameron and McAllister, 2016, Barton and Hart, 2001, Barton, 2000). The data presented within the following chapters represents the first exploration of AMR pathogens within peri-urban wild dogs.

Hookworm

In Australia, the species of canine hookworm include: *Ancylostoma caninum*; *A. braziliense*; *A. ceylanicum*; and *Uncinaria stenocephala*. Hookworm infection in humans occurs through percutaneous exposure through contact with ensheathed third stage larvae in the soil, oral exposure

to soil-contaminated water, food and possibly infected meat (Bowman et al., 2010). In north-eastern Australia, *A. caninum* is regarded as a major contributor to eosinophilic enteritis in humans (Prociv & Croese, 1990), which is characterised by clinical symptoms of abdominal pain, with or without blood, and eosinophilia. Infection can cause diarrhoea and weight loss or occasionally be subclinical (Croese et al., 1994b, Croese et al., 1994a). Human infection with hookworm is diagnosed through the identification of eggs in stool or based on classical lesions associated with cutaneous larva migrans, where the hookworm larva migrate underneath the skin leaving a visible red migration line, that is often itchy. Since *A. caninum* induced eosinophilic enteritis represents a non-patent infection, this avenue for diagnosis remains unsuitable (Prociv and Croese, 1996). Therefore presumptive diagnosis relies primarily on the patient's response to treatment with anthelmintics. Another drawback of coprological-based diagnosis of hookworms is that the species of infecting hookworm cannot be differentiated based on morphology of eggs alone. Recently in Western Australia, *A. ceylanicum* infection was reported in two individuals with no history of travel, representing the first autothonous cases in Australia (Koehler et al., 2013). This hookworm is of particular significance as it is the only one to produce patent infections in humans and is estimated to infect up to 100 million people (reviewed by Traub, 2013).

In canines, *A. caninum* is commonly transmitted vertically. Patent infections are caused in pups through infective larvae passed through the milk of bitches (Clapham, 1962). This can occur from either an acute infection or from arrested larvae that have become reactivated prior to parturition (Burke & Roberson 1985a, 1985b). This is a particularly important route of infection, contributing to maintenance of the pathogen within canine populations as the arrested larvae in the bitch can be a reservoir for infection for up to three litters (Kramer et al., 2009b, Traversa, 2012). In peri-urban wild dogs, this may be a significant factor in the ability of the pathogen to remain within the population, as dominant bitches are often difficult to trap (Glen Alchin, pers comm. 2016). Puppies infected via the transmammary route in turn shed *A. caninum* eggs in their faeces. This also leads to direct environmental contamination, which is a major source of infection in humans (McCarthy & Moore, 2000, Prociv et al., 1994). Current knowledge on the prevalence of hookworms in wild dog populations is not well understood, however the parasite is widely spread throughout tropical and subtropical Australia (Prociv et al., 1994). Seven percent of domestic dogs in Australia were infected with hookworm, and 70.7% of those were singular infections of *A. caninum* (Palmer et al., 2007). Practical methods to reduce environmental egg levels include anthelmintic treatment of pets, along with rapid removal of faeces from domestic and public areas. Breeding bitches can also be treated with larvacidal drugs (moxidectin, selamectin, fenbendazole) during pregnancy and lactation to block vertical transmission of infection to the pups (Burke & Roberson, 1983, Kramer et al.,

2009a, Payne-Johnson et al., 2000). However, the unmanaged presence of the pathogen in wild dogs and foxes can hinder these forms of control (Deplazes et al., 2011). Effective control in the wild requires reducing the number of infected young dogs (and foxes) through animal management or population control programs (Overgaauw & van Knapen, 2013).

Methicillin resistant Staphylococcus aureus

Staphylococcus aureus is a Gram positive coccus, found most commonly within the nasal passages and on the epidermis of humans and animals. Pathogenic strains often present as skin and / or soft tissue wound infections in humans and animals (Munckhof et al., 2003). Infection in humans can often progress to bacteraemia presenting as clinical signs associated with endocarditis, osteomyelitis and septic arthritis with the potential for other clinical signs dependent on the strain (Collignon et al., 2005; Nimmo and Playford, 2003). Treatment methods are often complicated because of antimicrobial resistance, that developed soon after the introduction of antibiotics with ongoing adaptation to later generations of antimicrobial classes (Collignon et al., 2005). Resistance to methicillin is indicative of loss of susceptibility to the common β lactam drugs that were the mainstay of *S. aureus* treatment, therefore indicating strains that significantly increase the complexity of treatment and management. Historically, MRSA was known as a hospital acquired infection, however, community acquired strains began becoming apparent in Australia in the late 1980's and have become increasingly responsible for causing disease in healthy individuals (Munckhof et al., 2003).

S. aureus has been detected in domestic dogs, with the proportion of isolates displaying antibiotic resistance significantly higher in dogs than humans (Boost et al., 2008). Several clonal strains of *S. aureus* have been identified among humans, livestock and companion animals, including a dog specific clonal complex. However, dogs are commonly infected with lineages of human origin (e.g. ST22 and ST5) (Malik et al., 2006; Strommenger et al., 2006; Weese et al., 2006). As a result, close contact with humans is typically associated with infection in domestic pets (Bierowiec et al., 2016). Other risk factors for MRSA infection in canines include previous receipt of antimicrobial drugs (Faires et al., 2010). To date, there have been no studies conducted to survey if *S. aureus* (both methicillin sensitive and / or resistant) is present within wild dog populations in Australia. Foxes in the UK were not found not to harbour MRSA, however, methicillin resistant non-aureus spp. staphylococci were detected (Carson et al., 2012). The public health significance of MRSA warrants future investigation into the role peri-urban wild dogs could potentially play in contribution towards community acquired MRSA.

Salmonella spp.

Salmonella spp. are another leading cause of bacterial gastroenteritis throughout the world (Majowicz et al., 2010). The most common non typhoid (and therefore zoonotic) serovars found in humans globally are *S. enterica* serovars Typhimurium and Enteritidis (Andino and Hanning, 2015). In Australia, Typhimurium is the most frequently reported serovar, representing 41% of all infections in 2009 (OzFoodNet., 2009). In Australia, most infections are likely due to overseas travel, but serovars Virchow and Saintpaul are more commonly reported, especially in the northern regions (Hendriksen et al., 2011; OzFoodNet., 2009). In Queensland, Virchow is the most frequently reported serovar, mainly seen in children around two to three months old (Callaway et al., 2011). In 2009 there were 9,533 notifications of infections with *Salmonella*, translating to 43.6 cases per 100,000 people (OzFoodNet., 2009). Humans become infected with *Salmonella spp.* commonly through ingestion of the bacteria from contaminated food or water but, also through direct or indirect contact with infected animal or human faeces (Andino and Hanning, 2015). The most common animal source of *Salmonella spp.* is poultry. However, livestock species, wildlife, and domestic pets are also potential sources of infection.

In Australia, there is limited published data about the presence of *Salmonella spp.* in dogs. In Brisbane, (Frost et al., 1969) found 6.9% of refuge, working, and veterinary clinic dogs to be infected with at least one serovar of *Salmonella spp.*, which around that time was a similar occurrence to the prevalence in the human population. The prevalence of *Salmonella spp.* in humans appears to have recently increased (The OzFoodNet Working Group, 2009, 2011) but there is no data available from dogs to suggest that a similar trend has occurred. A single wild dog scat was opportunistically found to be carrying *Salmonella spp.* although the species or serovar was not identified (Allen, 2006). The lack of information regarding the presence of *Salmonella spp.* in wild dogs again makes it difficult to address the potential for them to contribute as environmental reservoirs. So, it is unknown if wild dogs or other feral animals have become important in harbouring the pathogen.

Spirometra erinacei

Spirometra erinacei is a tapeworm, with definitive hosts being felines and canines. The parasite requires larval pleurocercoids to develop within copepods and other subsequently aquatic paratenic hosts (e.g. toads, frogs, snakes etc.) to continue the lifecycle (Lee et al., 1990). Infection in humans (known as sparganosis) generally occurs from ingesting uncooked mammalian, reptilian or amphibian meat where pleurocercoids migrate into tissues, which may localise in vital organisms such as an eye, the spinal cord, or brain (Kudesia et al., 1998; Li et al.).

Dogs are not directly involved in infecting humans, however, it is possible they play a significant role in the maintenance of the parasite within the environment, particularly within the wild boar population. *S. erinacei* is highly prevalent within the wild boar population in northern Australia (Pavlov et al., 1992) and is a valuable meat consumed amongst aboriginal communities (Koichi et al., 2012) and represents a valuable export commodity for Australia. Consumption by the general Australian public is likely limited, but despite this, *S. erinacei* remains a pathogen of interest due to its previously reported high prevalence rates within wild dogs (Jenkins et al., 2008).

Toxocara canis

Dogs and foxes act as major sources of toxocariasis, which is one the most highly reported zoonotic helminth infections in humans (Hotez and Wilkins, 2009; Macpherson, 2013). Humans act as paratenic hosts and become infected with *T. canis* upon the ingestion of embryonated eggs which have been shed in dog faeces and contaminate the environment (Overgaaauw, 1997). Once ingested, the larvae hatch and enter the circulatory system, migrate to the liver and lungs, and remain in the tissues as infective encapsulated larvae where they may remain viable for years. Another possible source of infection in humans is through consumption of L3s in the raw or undercooked muscle tissues of a paratenic host. Infections in humans may occasionally go un-noticed due to the absence of clinical signs, however syndromes that are associated with the pathogen include visceral larva migrans (VLM), ocular larva migrans (OLM), neurological toxocarosis (NT) and covert toxocarosis (CT) (Fan et al., 2013; Rubinsky-Elefant et al., 2010). VLM is associated with higher intensity of worm burdens and migration of larvae, being more common in children below eight years of age. Classical signs include fever, hepatomegaly, abdominal pain, vomiting, diarrhoea, respiratory signs such as coughing and wheezing, loss of appetite, and fatigue. OLM is generally associated with low worm burdens where the L3 migrate to the eye causing varying degrees of visual impairment. Neurotoxocariosis is associated with L3 migration to the spinal cord and brain resulting in meningitis, encephalitis, myelitis, cerebral vasculitis and seizures (Eberhardt et al., 2005, Vidal et al., 2003).

In Australia, *Toxocara canis* has been detected in low levels amongst populations of farm dogs (1.6% – 6.1%) (Jenkins et al., 2014a), pound dogs (2.4% -2.8%) (Jenkins et al., 2014a; Palmer et al., 2008), domestic dogs (0.4%) (Palmer et al., 2008), as well as peri-urban wild dogs (4.6%) (Jenkins et al., 2008). The low prevalence within domestic and farm dogs is likely related to anthelmintic use, however, the low prevalence within wild dogs suggests there may be a lack of paratenic hosts within the diet, or alternatively a lack of infective eggs within the environment in

general. Regardless of the low infection rates, the current and future infection status of wild dogs with *T. canis* remains an important pathogen to monitor due to the potential for significant public health impacts. The pathogen should also be monitored within the urban fox population.

Rickettsia spp.

Pathogens that infect dogs and/or humans via arthropod vectors are known as canine vector borne diseases (CVBDs). There are numerous pathogens that can be transmitted to dogs through the bites of ticks, fleas or other arthropod vectors (Hii et al., 2012) however only a limited number cause significant disease in humans. Rickettsial pathogens in Australia cause a variety of diseases in humans, dependent on the bacterial species involved. For example, *Rickettsia felis*, the causative agent of flea-borne spotted fever (FSF), is an emerging public health concern (Perez-Osorio et al., 2008). Symptoms in humans can range from non-specific flu like illness (fever, myalgia and headache) to severe multi-systemic disease owing to disseminate vasculitis (Reif & Macaluso, 2009, Zavala-Castro et al., 2009). *R. felis* is vectored by fleas, most commonly the cat flea *Ctenocephalides felis*. The purchase of two flea infested kittens led to the first Australian human outbreak amongst a family of five in Victoria (Williams et al., 2011). Data on *R. felis* infection in fleas revealed a 19.8% prevalence across eastern Australia, however infection of a feline host is yet to be detected (Barrs et al., 2010). Despite *R. felis* being able to maintain infection through transovarial and transstadial methods in flea vectors, the potential for canines to act as a mammalian reservoir for *R. felis* is a relatively new discovery in Australia (Azad et al., 1992). Nine percent of pound dogs tested in south east Queensland and three indigenous community dogs tested in the Northern Territory had detectable *R. felis* in their blood, providing strong evidence for dogs to be a potential reservoir for the pathogen (Hii et al., 2011b, Hii et al., 2011a). Populations of wild dogs or dingoes around Australia have yet to be tested for any rickettsial pathogens of zoonotic importance. However, considering the presence of the bacteria in community and pound dogs, it is likely that wild dogs could also harbour the pathogen.

INDIVIDUAL LEVEL FACTORS INFLUENCING PATHOGEN CARRIAGE IN PERI-URBAN WILD DOGS

Age

In general, younger canines appear to be more susceptible to infectious pathogens than their adult counterparts. Several studies have documented this occurring in domestic dogs. For *Campylobacter spp.*, dogs less than one year old are more susceptible (Hald et al., 2004, Sandberg et al., 2002, Westgarth et al., 2009). For parasites, the risk of infection also tends to decrease as dogs get older (Nijse et al., 2015, Visco et al., 1977). Canines under six months of age generally are more prone

to infection with *T. canis* (Katagiri & Oliveira-Sequeira, 2008), *Giardia duodenalis* (Bouzig et al., 2015), and hookworms (Palmer et al., 2008). Despite this, it has been suggested that age does not provide absolute immunity to hookworm and *Giardia* infections, but rather young puppies are more likely to shed eggs and cysts, respectively, at a higher intensity than adults (Traub et al., 2009). This may be a positive factor in wild dog infectious disease control, as younger dogs are often easier to capture due to their inexperience and also their requirement for movement whilst searching for a mate or new territory (Glen Alchin, pers comm). However, in some circumstances risk of infections in canines can increase with age, as may be the case for neosporosis (Paradies et al., 2007). Data offering conclusions on age related susceptibility to infectious agents in wild dogs is lacking.

Sex

Generally, males of any species are more susceptible to infectious pathogens than females (Coggins, 1998). It is thought that both ecological and physical (hormonal) differences between males and females contribute to the varying infection rates among sexes (Kramer et al., 2009a). Male wild dogs typically have larger home ranges than their female counterparts, particularly during the breeding season when the bitch may travel less, and also during mating season when males disperse from their pack to find a mate (Thomson, 1992). Hence, males may expose themselves to additional landscapes, with different resources and overlapping home ranges with other dogs and animals, and as a result, have greater exposure to an infected source. Despite differences in ecological behaviours, even under controlled laboratory conditions, males still have a higher prevalence of infections due to the differences in sex hormones (see Table 1. Payne-Johnson et al., 2000). Females may be able to better control specific pathogens once infected (Payne-Johnson et al., 2000). These results are generally explained through the action of oestrogen enhancing both cell-mediated and humoral immune responses, while testosterone suppresses the immune response (Klein, 2000). Higher levels of *T. canis* infection in dogs have been linked to males in several studies (Altizer et al., 2006, Stromberg, 1997). Male dogs in India were 10.5 times more likely to be infected with the roundworm over their female counterparts (Traub et al., 2005). Males have also been positively linked with carriage of *Ancylostoma spp.*, however this was not significant (Altizer et al., 2006). Although there are many infections where males are more susceptible, there are a few exceptions to the rule (Kramer et al., 2009b). During pregnancy, females tend to become less resistant to certain pathogens (McCarthy & Moore, 2000). Wild dogs may have a significant ability to disperse pathogens throughout the landscape, particularly when coupled with the ecological differences with which gender is associated.

ENVIRONMENTAL FACTORS INFLUENCING PATHOGEN CARRIAGE IN PERI-URBAN WILD DOGS

Physical environment

Land use and urbanisation can influence the amount of exposure wild dogs have to contaminated areas and infected individuals of other species. Foxes in the United Kingdom were found to have varying rates of cestode and trematode infections according to their rural or urban lifestyles (Richards et al., 1995). Contamination levels of soil by *Toxocara spp.* eggs have been shown to be negatively influenced by the presence of vegetation, and egg viability was also negatively influenced by increased presence of clay or sand in the soil (Cells Trejo et al., 2012). There has been limited research in Australia to compare environmental influences on pathogen presence. Foxes in Western Australia were more likely to be infected with the roundworm *T. canis* and hookworm *U. stenocephala* in the presence of native vegetation (Dybing et al., 2013). Hence there are likely to be numerous factors influencing survival of infectious stages of parasites in different environments. Peri-urban wild dogs on the Queensland Sunshine Coast were found to spend equal time in bushland and cane land at 38% each with the remaining 24% spent in urban areas (Allen et al., 2013). Individual dogs varied in their times spent within each sector, suggesting that peri-urban wild dogs are likely flexible in their resource selections. It further suggests that individual dogs have unequal exposure to the different ecological sectors and hence exposure to contaminated environments cannot be assumed to be equal for each wild dog.

Climate

It is well known that varying climatic conditions can influence the incidence of infectious pathogens in animals (Gordon, 1948). Climatic conditions can influence not only presence of infection in animals but also their ability to shed and disseminate the pathogen, which is important to maintain the enzootic cycle of infectious disease. Often, the lifecycle of parasites involves eggs developing in the environment to their L2 stage (e.g. *Toxocara canis*) or L3 stage (e.g. *A. caninum*) before infecting an intermediate host (Macpherson, 2013). This stage can be influenced by seasonal variation in temperature (Azam et al., 2012, Gamboa, 2005), humidity (Dybing et al., 2013), rainfall (Dybing et al., 2013), moisture (Gamboa, 2005), and wind. *A. caninum* exhibits seasonal variation due to hypobiosis allowing it to synchronise its life cycle to changes in its environment, providing it with a major advantage over other hookworm species in dry weather (Gibbs, 1982). As a result, specific parasites have seasonality peaks in annual cycles, possibly explaining the varying rates of infection across countries, states and regions. Bacteria and viruses can also be influenced by variation in humidity, temperature, and rainfall (Bi et al., 2008, Harris et al., 2013). Different characteristics of eggs, cysts and oocysts also have an important role in their ability to remain

infective in the environment under variable climatic conditions. Protozoan cysts and oocysts are prone to desiccation and viability can be highly influenced by temperature range and soil properties (Peng et al., 2008). Higher temperatures (up to 37 °C) significantly increases the rate of oocyst inactivation (King et al., 2005) and the presence of clay in the soil also was found to significantly decrease survival rates for some oocysts (Peng et al., 2008). In comparison, thick-walled eggs such as *Toxocara spp.* and *Taenia spp.* are highly resistant to adverse climatic effects. *T. canis* eggs have also been shown to survive in soil for extended periods with temperature and humidity having a significant effect on development rates (Gamboa, 2005). The optimum conditions for development are thought to be around 30 °C and in moist soil because higher temperatures up to (>37 °C) in laboratory conditions inhibited embryonation (Catling et al., 1992). Although laboratory experiments provide guidelines for optimum conditions, external environmental factors can often influence these results. Eggs of *E. granulosus* were still infective to ovines after 41 months of being exposed to natural environmental conditions classified as an inferior arid climate (Thevenet et al., 2005). Along with climatic effects on pathogen presence and infectivity, wild dog movements and habitats may also differ seasonally, as the breeding season and migration can alter their travel patterns (Thomson, 1992). This is likely to influence spread of disease at those times, either possibly expanding pathogens to new areas or increasing intensity within a location.

APPROACHES TO CONTROL

Typical approaches to wild dog control

Like other vertebrate pests, current wild dog control programs are generally based around reducing or managing impacts through culling programs (Fleming et al., 2014). Land producers, contracted pest controllers or local government officers may bait, trap and/or shoot proactively or reactively because of wild dog damage to livestock, wildlife, or because of potential risks to the public. Usually, the strategies and methods used for broad scale rural wild dog control cannot be directly transferred into the peri-urban and urban environments due to legislative restrictions and risks to domestic pets. As a result, control of these wild dog populations is restricted, and reduced in space and time.

Control in peri-urban environments

Traditional control methods, such as baiting, are restricted by state regulations that generally inhibit the use of toxins close to residential areas (APVMA, 2008). In Queensland, it is a legal requirement to notify all residents whose property boundary is within one kilometre of the bait site 72 hours prior to baiting (DAF., 2017). In addition, baits cannot be laid within five kilometres of a town without biosecurity approval. In peri-urban areas this can relate to an extremely high number of

residents and often becomes too difficult, making baiting unfeasible. Secondly, wild dog and human interactions pose direct risk to human and / or domestic animal health safety but also pose a potential risk towards public health, through the spread of zoonotic pathogens. Although there has been some opportunistic data collected regarding wild dog pathogens, epidemiological studies and a true understanding to the zoonotic potential are lacking.

Control for infectious disease prevention

There is a lack of information on the impact of wild dog population control on the intensity, prevalence and transmission of pathogens. Considering many of the zoonotic diseases discussed above are not notifiable under government guidelines (NNDSS, 2013), limited information relating to human infection also makes the decision of where and when to implement control measures difficult. In some cases, eggs of pathogens (i.e. *Taenia spp.*, *Toxocara canis*) are highly environmentally resistant and there are no practical methods available for reduction of egg levels within the environment (Overgaauw and van Knapen, 2013). Hence the most effective management protocols should attempt to reduce or prevent the initial environmental contamination. Reducing the overall population density is a common method used for managing disease in wild animals (Fullerton et al., 2007). However, considering an individual dog may excrete in excess of 500,000 *N. caninum* oocysts, it is possible that reducing the numbers of wild dogs *per se* may not reduce the transmission rate (Gondim et al., 2002). Hence, current control methods may not impact on pathogen intensity, prevalence and/or transmission potential because it is impossible to specifically target infected canines with high intensity infections. This could also be true for *E. granulosus* with five of 50 infected wild dogs responsible for harbouring 66.4% of total worms recovered (Jenkins et al., 2008). However, praziquantel baiting for *Echinococcus spp.* has been shown to be effective in reducing the prevalence of *E. multilocularis* in urban fox (and the intermediate host) populations across the globe (Hegglin et al., 2003, Takahashi et al., 2013). Significant reductions in the tapeworm are seen when regular baiting is imposed, however baiting is required to be conducted frequently (Hegglin & Deplazes, 2013). Hence, it needs to be continued over several years to become cost effective as well as targeted in high endemic areas close to human populations where it can have the most significant impact. Although specific bait uptake studies for praziquantel have not been conducted in Australia for foxes nor wild dogs, both species will take and can be managed effectively with toxic baits (Fleming et al., 2014). Studies have assessed bait uptake by foxes for rabies contingency planning (Fleming, 1997, Marks & Bloomfield, 1999) and suggest that baiting for rabies could be a feasible option. Targeted baiting with praziquantel could be a viable control method for reduction of *E. granulosus* in Australia although studies need to be conducted to assess optimal baiting intensity (e.g. baits per square kilometre, distribution patterns) and address any non-

target species concerns, particularly in peri-urban areas. However, along with focusing on reduced environmental contamination, it should be combined with education, especially in peri-urban regions, with a focus on personal hygiene and responsible pet ownership.

As mentioned throughout this review, there are numerous factors that can influence the presence, intensity, and prevalence of many pathogens. Using geographical information systems (GIS) allows for the visualisation and detection of patterns (if any) of pathogens, identifying important clusters, environmental risks or other unusual influencing factors impacting on the distribution of pathogens. Disease modelling is also beneficial for generating important information regarding potential spread of disease and allows for more informed intervention choices to be made. More detailed information about the methods and applications of spatial ecology and epidemiology can be found in any one of several reviews published (Auchincloss et al., 2012, Carpenter, 2001, Gatrell et al., 1996, Pullan et al., 2012, Ward & Carpenter, 2000). The implementation of these methods into wild dog management programs can assist to highlight the key locations for targeted control of pathogens that harboured amongst peri-urban wild dog populations.

CONCLUSIONS

Detailed information of peri-urban wild dog pathogens is limited in Australia, although they do harbour potentially significant pathogens of public health and/or economic impact. There is a strong need for more knowledge on pathogen prevalence, clustering of disease, and risk factors as well as how this relates to general peri-urban dog ecology such as density, habitat use, home-range studies, and demographics of wild dog populations. This will enable a more holistic understanding of the role of these wild canines in pathogen carriage and spread, and impacts on human and livestock health. Moreover, such knowledge can be used to define appropriate management strategies to reduce the impacts of these pathogens. Current control methods for peri-urban wild dogs and foxes are likely insufficient in managing disease, however, limited information on human infection means that the impact on public health is currently unknown.

CHAPTER 3

The presence of zoonotic and/or economically significant pathogens of peri-urban wild dogs across north east New South Wales and south east Queensland

ABSTRACT

Wild dogs are common in Australia and are relatively frequent within peri-urban regions, particularly along the eastern seaboard. They are known to harbour a variety of pathogens, some of which present as a public health concern, or can cause disease and economic losses within the livestock industry. This study conducted a cross sectional investigation into a broad range of parasitic and bacterial pathogens of 201 peri-urban wild dogs from south-east Queensland and northern New South Wales. Helminth parasites were detected within 79.6% of peri-urban wild dogs. *Echinococcus granulosus* was the most common pathogen, with adult worms detected within 50.7 ± 6.9 % of intestines, followed by *Spirometra erinacei* (36.6 ± 6.4 %); Hookworms, including *Ancylostoma caninum* and *Uncinaria stenocephala* (28.8 ± 7.1 %); *Toxocara canis* (5.4 ± 3.1 %) and *Taenia spp* including *T. serialis* and *T. pisiformis* (4.5 ± 2.8 %). Bacterial pathogens detected included methicillin resistant *Escherichia coli* (20 ± 10.1 %), *Salmonella spp* (3.7%) and methicillin sensitive staphylococcus aureus (3.3 ± 2.7 %). Our results have provided essential baseline data into the presence of pathogens in peri-urban wild dog populations in north-eastern Australia. This information can be used to identify, inform and refine further research into significant pathogens. It also enables future recording of the status of pathogens in peri-urban wild dogs, which can be used for comparison in monitoring studies. This is essential to inform the management of wild dog impacts in peri-urban areas.

INTRODUCTION

Successful adaption to peri-urban and urban habitats around the globe has been achieved by a number of wildlife species (Koenig et al., 2001; Prange et al., 2003; Statham and Statham, 1997). Although a clear definition of peri-urban is difficult to obtain it is commonly classified as a transitional zone between urban and rural landscapes (Low Choy et al., 2007). In Australia, wild dogs including: dingoes (*Canis lupus dingo*); feral domestic dogs (*Canis lupus familiaris*); and their cross breeds (*C. l. dingo* x *C. l. familiaris*) inhabit the majority of the mainland (Fleming et al., 2014). This includes most of the major capitals, as well as other cities and towns, especially along the eastern seaboard (Allen et al., 2013). Anecdotally, within the past ten years, wild dog numbers in peri-urban areas of Australia have become gradually more abundant. This is particularly true for South East Queensland (SEQ) where increased reports, complaints and captures over recent years suggest they are now relatively common (Glen Alchin, pers comm). Human settlement areas in SEQ are often surrounded by remnant patches of vegetation and cropping land. These provide the perfect corridor for wild dog interaction with human resources.

Urban-adapted species generally exhibit much smaller home ranges than their rural counterparts. This was found to be true for peri-urban wild dogs, that were also shown to commonly utilise public environments through traversing parklands, school yards and other built up areas including residential backyards (Allen et al., 2013). This is concerning not only for potential human and wildlife conflicts including attacks on the public, their pets, livestock animals, and native wildlife but also introduces the potential for transmission of zoonotic pathogens. The significance of peri-urban wild dogs in harbouring zoonotic pathogens is currently unknown. Previous research has largely focused on domestic household, rural working and/or pound populations of animals (Jenkins et al., 2014a; Palmer et al., 2008). For many of the common pathogens, the epidemiology, modes of transmission, routes of infection, parasite life cycles and pathogenicity of their infections are relatively well described (Chen et al., 2012; Deplazes et al., 2011). However, the significance of infection in domestic canines often is reduced by the fact that most animals are treated with broad-scale anthelmintic therapy. In Australia, this is also assisted by the requirement of owners to remove any faeces left by their dog in public use areas. This usually happens immediately and is assisted by the availability of garbage bags and disposal bins at most dog friendly parks. As a result, contamination of environmental sources with infectious pathogens is more likely to be attributed to untreated wild populations of animals. Peri-urban wild dogs should therefore be considered an important factor in the potential for disease transmission to humans but there also remains a need to consider economic costs on production industries. Peri-urban regions play a significant role in agricultural production around Australia (Choy and Buxton, 2013). Some pathogens are significant

to both humans and livestock but many are specific to only one group. It remains important to consider both the human health aspects and potential economic impacts on the livestock industry from peri-urban wild dogs.

The general knowledge surrounding the presence or prevalence of significant pathogens in peri-urban wild dog populations is severely lacking. Previous research into pathogens carried by wild dogs has mostly focused on presence or prevalence of specific pathogens such as *Echinococcus granulosus* and much of the data has been collected opportunistically. Despite highlighting the potential impacts on human health, detailed epidemiological studies focusing on wild dog populations have not been conducted, and remain sorely needed.

The aim of this study was to conduct a cross sectional investigation into the proportion of zoonotic pathogens harboured by peri-urban wild dogs in SEQ with significant potential for public health risk and/or economic risk to livestock industries. Beyond providing valuable estimates of infection burden, this knowledge is essential to identify, inform and refine further, more detailed studies into significant pathogens.

MATERIALS AND METHODS

Study population

Two hundred and one whole wild dog carcasses were supplied between August 2012 and May 2015. Males and females were equally represented within the data set at 49% and 51%, respectively. Peri-urban wild dogs provided for this study were trapped either as a part of routine council control programs or private pest management programs. Trapping locations were often influenced by public complaints or existent active management plans for control of feral animals. Wild dogs were either caught with foothold traps and shot or were shot opportunistically by contracted hunters. They were captured from South East Queensland (SEQ), a small section of Northern New South Wales, and regions just north of SEQ such as the Gympie local government area (LGA) (Figure 3.1). LGA's included: Bundaberg Regional Council; North Burnett Regional Council; Gympie Regional Council; South Burnett Regional Council; Somerset Regional Council; Sunshine Coast Council; Moreton Bay Regional Council; Ipswich City Council; Scenic Rim Regional Council; Logan City Council; Brisbane City Council; Gold Coast City Council; Byron Bay Regional Council; and Lismore City Council. Ethical approval for the study was provided by the University of Queensland Animal Ethics Committee (approval number SVS/145/13).

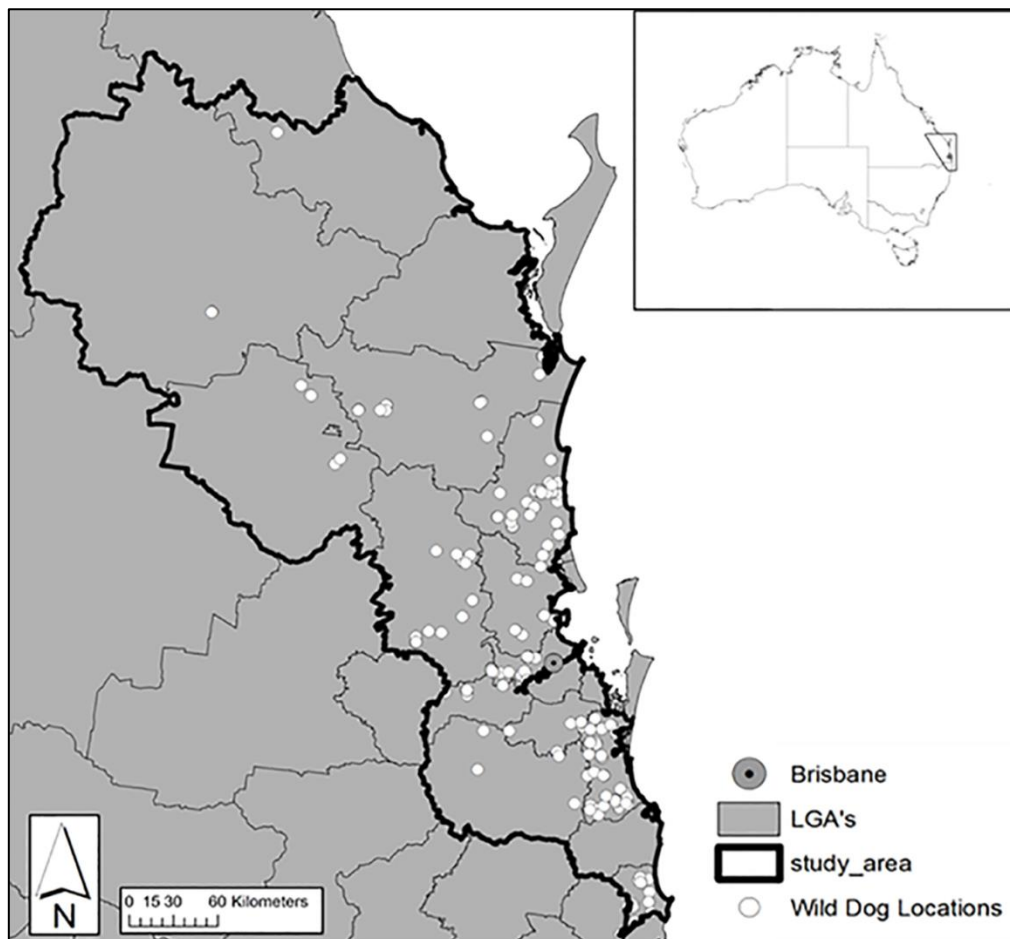


Figure 3.1 Geographical locations of the 201 peri-urban wild dogs captured during routine management programs and supplied for this study

Sample collection

Exposure and/or identification of zoonotic pathogens were conducted using blood and stool samples combined with examination of intestinal tracts. Blood samples were collected via cardiac puncture and transferred to ethylenediaminetetraacetic acid (EDTA) and serum-separator (SST) vials (Becton, Dickinson and Company, Australia) and kept cool. Where possible, supernatant was collected off the SST tubes and stored frozen with the EDTA sample. Faecal samples were collected from the rectum and stored in three separate tubes containing one each of: 10% formalin; 2.5% potassium dichromate; and 20% glycerol. Faeces in formalin and potassium dichromate were stored at room temperature and faeces in glycerol were stored at -80 °C. After samples had been collected, wild dog cadavers were placed into large body bags and frozen at -18°C for later necropsy. At necropsy, the trachea was clamped before removing the heart and lungs. A random selection of 40 wild dogs was assessed for heartworm (*Dirofilaria immitis*). An incision was made along the coronary artery to expose the right atrium and ventricle. The right pulmonary artery was followed into the cranial, medial and caudal lobes of the right lung. The entire small intestine was removed

from all dogs (n=201) and its contents were flushed with water and expelled into a dish. The intestinal contents were then transferred into a disposable container for transport to the laboratory and kept refrigerated (3°C) until examination within 24 hours. The intestinal tract was then slit longitudinally and the mucosa examined for the presence of hookworms or other parasites. The top jaw was removed utilising a hack saw for the purpose of collecting the top two canine teeth to age the wild dogs for future studies. Throughout this process the nasal cavities were assessed for the presence of tongue worm *Linguatula serrata*.

Laboratory procedures for the identification of zoonotic pathogens

Parasite isolation and identification

Intestinal contents were filtered initially thorough a 918 µm sieve and washed into a beaker to isolate larger parasites. Parasites were placed into a petri-dish for initial identification and then stored in 70% ethanol. All adult helminths were identified based on morphological characteristics (Bowman, 2014). Contents of the beaker were then washed through a 200 µm fine sieve. All items trapped on the mesh were washed into a clean beaker and the total volume adjusted to 1000 ml. Contents were stirred and two 50 ml subsamples were collected. Each subsample was individually examined in small amounts under a dissecting microscope in a petri-dish marked with approximately 1 cm squares. Parasites other than those discovered in the coarse sieving stage were identified. For the case of *Echinococcus granulosus*, worms were counted for two 50 ml subsamples. As previously described by Jenkins et al. (2008), the sum of worms was multiplied by ten to provide an estimate of the total *E. granulosus* worm burden for each dog. Faecal samples in 10% formalin (n=156) were washed and subject to sodium nitrate (S.G 1.20) (Inpankaew et al., 2014b) and zinc sulphate (SG1.18) (Faust et al., 1938) flotation for egg/oocyte identification and quantification. The entire coverslip was examined under a light microscope at 10X and 40X magnification and the difference species of eggs/oocytes on the slide were noted and the total number of each species was counted. The total numbers of eggs were multiplied by a factor, dependent on the washed faecal pellet size (often X5) to provide eggs per gram of faeces.

Genomic DNA extraction from faecal samples

Aliquots of approximately 2 g of faeces were washed twice with PBS and centrifuged at 2000 g for three minutes, removing the supernatant each time, to remove traces of potassium dichromate. A 200 mg subsample of faeces was transferred to an empty 2 ml tube with approximately 1 g of silica/zirconia 0.5 mm beads (Daintree Scientific, Tasmania, Australia) and 370 µl of Mo Bio Powerbead solution from the PowerSoil DNA isolation kit (Mo Bio Laboratories Inc., California,

United States of America) The remaining DNA extraction protocol was performed as per the Mo Bio protocol (<https://mobio.com/media/wysiwyg/pdfs/protocols/12888.pdf>) with minor modifications. Fifty µl of C6 solution was used at step 20 to increase DNA concentration. DNA concentration was measured using a nano drop 1000 spectrophotometer (Thermo Fisher, Massachusetts, United States of America).

Genomic DNA extraction from blood samples

DNA was extracted from whole-blood samples collected in EDTA tubes. The DNeasy Blood and tissue kit (Qiagen, Hilden, Germany) was used following the manufacturer's protocol with only minor modifications. The blood volume was increased to 200 µl and the final elution volume was decreased to 50 µl for more concentrated DNA. DNA concentration was measured and adjusted (if needed) to approximately 50 ng prior to PCR.

Genomic DNA extraction from worms

DNA was extracted from *Taenia* sp. adult worms, morphologically-identified according to (Bowman, 2014). The DNeasy Blood and Tissue kit (Qiagen) was used following the manufacturers protocol with minor modifications. Approximately 0.25 g of worm tissue was incubated at 56 °C with 180 µl ATL buffer and 20 µl of proteinase K until completely lysed. The final elution volume was decreased to 50 µl. DNA concentration was measured and adjusted (if needed) to approximately 50ng prior to PCR.

Polymerase Chain reaction (PCR)

Eukaryotic DNA

All DNA samples were screened for eukaryotic DNA to ensure the presence of amplifiable DNA in samples. Samples were tested using universal primers 18SEUDIR 5'-TCTGCCCTATCAACTTTCGATGG-3' and 18SEUINV 5'-TAATTTGCGCGCCTGCTG- 3' for amplification of 140 bp fragment of the nuclear 18S gene from eukaryotic DNA (Fajardo et al., 2008). The annealing temperature was modified to 60 °C (Wang et al., 2013).

Hookworms

Samples were tested using previously published primers, PCR reaction and cycling conditions to amplify a section of the internal transcribed spacer (ITS) regions of *A. caninum*, *A. ceylanicum*, *A. braziliense* and *U. stenocephala* (Traub et al., 2007; Traub et al., 2004). Amplified ITS PCR

products were subjected to direct digestions as described in Palmer et al. (2007) to differentiate between species. PCR products that were too faint to visualise using RFLP were submitted to the Animal Genetic Laboratory (School of Veterinary Science, University of Queensland) for PCR product purification and bidirectional DNA sequencing for species confirmation. DNA sequences were analysed on Finch TV 1.4.0 (Geospiza, 2009) and compared to published sequence data on GenBank using the basic logical alignment search tool (BLAST) (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). To produce an overall percentage of hookworm prevalence data the intestinal, faecal and PCR methods were combined. The data was refined to include only those which had faecal samples tested (either PCR and / or faecal float (n=156)). From the wild dogs that had associated faecal samples, a positive was accepted when an animal was positive in either intestinal, faecal float and / or PCR. (All wild dogs that had identified adult worms within their intestines were positive at faecal float and / or PCR). As a result, the overall prevalence presented is likely an under representation of the true prevalence.

Taenia

Primers F 5'-CATCATATGTTTACGGTTGG-3' and R 5' GACCCTAATGACATAACATAAT 3' were utilised to amplify of a 300-350 bp region of the *cox1* gene. Three microliters of extracted DNA from adult worms was added to a 22 µl reaction mixture containing 10 pmol of each primer, 12.5 µl of AmpliTaq Gold mastermix (Thermo Fisher) and a final volume of nuclease-free water. *Taenia* spp. DNA was used as a positive control and nuclease-free water as a negative. PCR amplifications were performed with the initial activation step at 95 °C for five minutes followed by 40 cycles of 95 °C for 30 seconds, 54 °C for 30 seconds, 72 °C for 30 seconds, and a final extension step on 72 °C for seven minutes. Amplified product was examined on a 1.5% agarose gel stained with SYBR Safe (Invitrogen, California, United States of America) run at 120V for 60 minutes. All samples were submitted to the Animal Genetic Laboratory (School of Veterinary Science, University of Queensland) for PCR product purification and bidirectional DNA sequencing for species confirmation. DNA sequences were analysed on Finch TV 1.4.0 (Geospiza, 2009) and compared to published sequence data on GenBank using BLAST.

Spotted Fever Group Rickettsia

Samples were tested using primers ompB-F 5'- CGACGTTAACGGTTTCTCATTCT-3' and ompB-R 5' ACCGGTTTCTTTGTAGTTTTCGTC-3' for amplification of a 297 bp region of the outer membrane protein B (*ompB*) gene of spotted fever group *Rickettsia* according to (Hii et al., 2011). Amplified product was examined on a 1.5% agarose gel stained with SYBR Safe (Invitrogen) run at 120 V for 60 minutes.

Neospora caninum

Samples were tested using previously published primers from (Fish et al., 2007). Three microliters of extracted DNA was added to a 23 μ l reaction mixture containing 10 pmol of each primer, 12.5 μ l of AmpliTaq gold master mix (Thermo Fisher), and a final volume of nuclease-free water. The cycling protocol comprised an initial activation step at 95 °C for five minutes, followed by 35 cycles of amplification at 95 °C for 15 seconds, 56 °C for 30 seconds, 72 °C for 45 seconds and a final extension step of 72 °C for seven minutes.

Isolation of bacterial species

Salmonella spp.

Faecal samples were stored in 20% Glycerol at -80 °C until testing. The faeces was swabbed and suspended in 5 ml of Rappaport-Vassiliadis (RV) broth (Oxoid, Cheshire, England) and incubated at 37 °C for 18-24 hours. Ten μ l of the enrichment broth was aspirated and streaked onto Brilliant Green Sulfa agar (BGA) to be incubated at 37 °C for 18-24 hours. Isolates represented by red-pink-white colonies surrounded by a red/pink zone were subcultured onto sheep blood agar (SBA) and MacConkey agar (MCA). All suspect *Salmonella* organisms were subject to a microbact™ test (Oxoid). Positive ID for salmonella were placed onto nutrient slopes and sent to Queensland Health Forensic and Scientific Services for serotyping.

Campylobacter spp.

Faecal samples were transferred using a sterile dry swab onto modified charcoal cefoperazone deoxycholate agar (Oxoid) and incubated in an atmosphere consisting of 5-6% oxygen, 10% carbon dioxide and 84-85% nitrogen at 41.5 °C for 48 hours.

Escherichia coli

Screening of samples focussed on identification of multi-drug resistant strains. Faecal samples were partially defrosted before using a dry swab and streaking onto Ampicillin- MacConkey (AMP-MCA) plates (Oxoid) and incubating at 37 °C for 18-24 hours. Three suspect colonies were selected and plated individually onto SBA plates which were again incubated for 18-24 hours at 37 °C. Growth on SBA plates were subject to rapID™ Spot Indole (Remel, California, United States of America) and Pyrrolidonyl Arylamidase (PYR) testing to confirm suspect colonies. Confirmed *E. coli* colonies were subjected to sensitivity testing. Three to four colonies confirmed as *E. coli* were

selected and incubated in 4 ml of Mueller-Hinton Broth at 37 °C for 3-4 hours or until denser than 0.5 McFarland standard. Incubated broth was added to 4 ml of saline until equivalent to a 0.5 McFarland standard. Two drops of the solution was dropped onto Mueller Hinton Agar (MH) and streaked evenly across the surface of the plate. Another drop was placed on SBA for purity testing. Antimicrobial disks were stamped onto the streaked MH plate. Both the MH and SBA plates were incubated at 37 °C for 18-24 hours. Chosen antimicrobials were commonly used in human or veterinary medicine and included: cephalothin (KF); amoxicillin -clavulanate 2:1 (AMC); ampicillin (AMP); cefoxitin (FOX); ceftazidime (CAZ); ciprofloxacin (CIP); trimethoprim-sulphamethoxazole (SXT); tet-racycline (TE); amikacin (AK); gentamicin (CN); tulathromycin (TUL); and florfenicol (FFC) (Oxoid Ltd).

Methicillin Resistant Staphylococcus aureus (MRSA) /Methicillin Sensitive Staphylococcus aureus (MSSA)

Nasal swabs were collected at necropsy and were suspended in 1.5-2.0 ml of Mueller-Hinton broth containing 20% (v/v) glycerol and stored at -80 °C. 100 µl of the broth was aspirated and added to 2.0 ml of Mueller-Hinton broth containing 6.5% (w/v) NaCl and incubated at 37 °C for 18-24 hours. To test for MSSA, 10 µl of broth was aspirated and streaked onto mannitol salt agar containing 6.5% (w/v) NaCl. Plates were incubated aerobically at 37 °C for 18-24 hours. Suspect MSSA colonies were subjected to a Gram stain, catalase test, and staph latex agglutination test for confirmation. To test for MRSA, 10 µl of broth was aspirated and added to 2-5 ml of tryptic soy broth containing 2.5% (w/v) NaCl, 3.5 mg/L cefoxitin and 20 mg/L aztreonam and incubated at 37 °C for 18-24 hours. 10 µl of the selective enrichment broth was aspirated and streaked into brilliance MRSA agar.

Serology

Serum samples were taken to the Queensland Department of Agriculture and Fisheries Biosecurity Sciences Laboratory, Coopers Plains (Queensland) for *Brucella suis* testing. Samples underwent an initial Rose Bengal (RB) test with brucellosis antigen (IDEXX, Maine, United States of America). Samples with the presence of agglutination were subjected to complement fixation (CF) testing for confirmation.

Statistical analysis (descriptive)

The percentage present and 95% confidence intervals (CI) were calculated for all pathogens within the sampled population. Percentage present was calculated by dividing the number of samples

positive for each pathogen by the total number of samples tested overall. The 95% confidence intervals were calculated by determining the standard error of the mean where: $CI = \hat{p} \pm z^* \left(\sqrt{\frac{\hat{p}(1-\hat{p})}{n}} \right)$

CI represents the 95% confidence interval, \hat{p} represents the population proportion, z represents the corresponding z value for the 95% confidence level and n is the sample size.

RESULTS

Recovery of zoonotic parasites

A total of 79.6% of dogs sampled had a parasitic infection and of those 28.1% were infected with multiple species (Table 3.1). Mixed infections consisted most commonly of *E. granulosus* ($n=40$) and *S. erinacei* ($n=29$). Two wild dogs were infected with three, and two were also infected with four different intestinal parasites. *E. granulosus* was the most common parasite detected in 50.7% (CI 43.8 – 57.7) of all dogs, followed by *S. erinacei* and *A. caninum* at 36.3% (CI 29.7-43.0) and 28.8% (CI 21.7-36.0), respectively. Only 5.5% of adult hookworms were identified within the intestinal tracts of the wild dogs at necropsy. Faecal floatation methods increased the prevalence to 17.2% and molecular methods further increased prevalence to 39%. All samples that were positive at faecal float, and had a corresponding sample for PCR were also positive at PCR. Several samples that were negative at faecal float returned a positive result at PCR. Taking into consideration the variations in sample size across the different testing methods, the overall combined hookworm prevalence was 28.8%. Four dogs were also shown to be infected with *Uncinaria stenocephala* and DNA sequences of these isolates showed 99% or greater similarity to previously published sequences of Genbank accession no. HQ262054.1 for *U. stenocephala*. 5.4% (CI 2.3 - 8.6) of wild dogs harboured *Toxocara canis* infections and 4.5% (CI 1.6 -7.3) of wild dogs were infected with *Taenia* spp. Of nine adult worms subjected to PCR and DNA sequencing, seven returned clean and readable sequences. BLAST results showed six of seven to have 100% sequence similarity to previously published sequences of Genbank accession no. AB731674.1 for *T. serialis* and one of seven to have 94% sequence similarity to previously published sequence of Genbank accession no. JN870104.1 for *T. pisiformis*. PCR did not detect *Neospora caninum*. No adult *D. immitis* or *L. serrata* were visualised on post-mortem examination.

Table 3.1 Number of wild dogs positive for parasitic pathogens for each sampling technique. *Gold standard testing method. ^PCR conducted on DNA from the adult worms not faecal sample.

Parasite	Samples positive in intestine (n=201) n (% \pm CI)	Samples positive by faecal float (n=156) n (% \pm CI)	Samples positive by PCR (n=82) n (% \pm CI)	Samples positive based on combined methods % \pm CI
<i>Echinococcus granulosus</i>	102 (50.7 \pm 6.9)*	7 (4.3 \pm 3.1)	n/a	50.7 \pm 6.9
Hookworms	11 (5.5 \pm 3.1)	28 (17.2 \pm 5.9)	32 (39.0 \pm 10.5)*	28.8 \pm 7.1
<i>A. caninum</i>			32 (39.0)	
<i>U. stenocephala</i>			4 (4.9)	
<i>Spirometra erinacei</i>	73 (36.3 \pm 6.4)*	42 (25.7 \pm 6.8)	n/a	36.6 \pm 6.4
<i>Taenia</i> spp.	9 (4.5 \pm 2.8)*	7 (4.3 \pm 3.1)	-	4.5 \pm 2.8
<i>T. serialis</i>			6 (3.0)^	
<i>T. pisiformis</i>			1 (0.5)^	
<i>Taenia</i> spp.			2 (1.0)^	
<i>Toxocara canis</i>	11 (5.4 \pm 3.1)*	7 (4.3)	n/a	5.4%

Bacteria

The bacterial pathogens of interest were not dominant within the faecal material and nasal passages of peri-urban wild dogs (Table 3.2). *Salmonella* spp. were found at low levels with ampicillin resistant *E. coli* being relatively more common. 20% (CI 9.9 – 30.1) of faeces sampled (n=60) contained ampicillin (AMP) resistant *E. coli*. Of those samples, 33.3% were only resistant to AMP, 41.6% were resistant to AMP plus one other antimicrobial and 25% were resistant to AMP plus two other antimicrobials. The additional antimicrobial that experienced resistance included: AMC; STX; TE; and/or KF. *Proteus mirabilis* was also identified in a single faecal sample. *Brucella suis* was not detected within the sampled population. Multiple samples (n=7) were suspect positive on the RB test they were all negative at CF. All samples were also negative for *Rickettsia felis* and methicillin resistant *S. aureus*, however low levels of methicillin sensitive *S. aureus* were detected.

Table 3.2 The presence of bacterial pathogens in peri-urban wild dogs. The number (n) of samples tested, and number (n) identified as positive, the region that positive samples were collected and the presence or absence of co-infections are shown.

Bacterial species	Samples tested n	Samples positive n (% \pm CI)	Region	Co infections
<i>Brucella suis</i>	39	0 (0)	-	-
<i>Campylobacter spp.</i>	82	0 (0)	-	-
Ampicillin resistant <i>Escherichia coli</i>	60	12 (20 \pm 10.1)	Gold Coast Gold Coast Gold Coast Gold Coast Sunshine Coast Somerset Somerset Somerset Byron Byron Byron Ipswich	<i>Echinococcus</i> , <i>Toxocara</i> , Hookworm <i>Echinococcus</i> , <i>Toxocara</i> <i>Echinococcus</i> <i>Hookworm</i> , <i>Spirometra</i> <i>Spirometra</i> <i>Echinococcus</i> , <i>Spirometra</i> <i>Spirometra</i> <i>Echinococcus</i> , Hookworm <i>Echinococcus</i> , MSSA <i>Echinococcus</i> <i>Echinococcus</i> , <i>Salmonella sp.</i>
MRSA	164	0 (0)	-	-
MSSA	164	5 (3.3 \pm 2.7)	Gold Coast Gold Coast Gold Coast Sunshine Coast Byron	<i>Echinococcus</i> , Hookworm <i>Echinococcus</i> , <i>Spirometra</i> <i>Echinococcus</i> Hookworm, <i>Spirometra</i> <i>Echinococcus</i> , <i>E. coli</i>
<i>Rickettsia felis</i>	82	0 (0)	-	-
<i>Salmonella spp.</i> <i>S. potsdam</i> <i>S. birkenhead</i> <i>S. subsp 1</i>	82	3 (3.7 \pm 4.0)	Ipswich Byron Gold Coast	<i>Echinococcus</i> , Hookworm, <i>E. coli</i> Hookworm Unknown Tapeworm

DISCUSSION

The results of this study indicate that parasitic infections in peri-urban wild dogs are common, with tapeworms representing the majority of intestinal pathogens. Furthermore, apart from an honour's report of a single wild dog with a *Salmonella* infection (Allen, 2006), this is the first report of bacterial testing in the Australian wild dog population. Previous studies looking at parasite carriage in wild dogs across Australia have reported high prevalence of pathogens such as *S. erinacei* (Coman, 1972; Jenkins et al., 2008), *Taenia spp.* (Coman, 1972; Jenkins et al., 2014b), and *E.*

granulosus (Baldock et al., 1985; Jenkins et al., 2008; Jenkins and Morris, 1991; Morrison et al., 1988). Using a geographically diverse sample of peri-urban wild dogs from SEQ and northern NSW this research demonstrates that *E. granulosus*, hookworms and *S. erinacei* can commonly be infecting these populations. This is in line with previous studies; however these results extend previous knowledge in that the majority of other research has been based on rural populations of wild dogs that are exposed to very different physical and climatic environmental conditions, and also exhibit differences in their home range territories (Allen et al., 2013). There are only two previous studies that report the presence of *E. granulosus* from an urban or peri-urban environment. The first, based on a sample size of 27 wild dogs and located in the Northern Queensland town of Townsville reported a prevalence of 22% (Brown and Copeman, 2003). The second, based on a sample size of 108 wild dogs and located in the Maroochy region of SEQ reported a prevalence of 46.3% (Jenkins et al., 2008). A third study located near Cairns did not detect any *E. granulosus* from 26 wild dog intestines (Smout et al., 2013). Climatic factors such as high temperatures and increased humidity are likely major factors in the reduced prevalence and intensity of *E. granulosus* in northern Australia (Torgerson and Heath, 2003; Wachira et al., 1991). Although the Maroochy study reported a somewhat similar prevalence as this study, all of the infected dogs were located towards the north and western regions, with those closest to residential areas free of the pathogen.

The data presented in this thesis shows that *E. granulosus* is not limited to a particular region but is common across the entire of SEQ, including parts of north-eastern NSW, which exhibits similar climatic conditions. This is a significant concern for public health. SEQ is a highly populated area, home to approximately 66% of the State's total population, being resident to an estimated 3.4 million people (ABS, 2011). Within the study area, wild dogs have been shown to reside within 700 m of residential houses at all times, to cross roads, pass through suburban backyards and traverse through frequently utilised public areas such as school yards and parklands (Allen et al., 2013). Unlike some other parasitic pathogens, the eggs of *E. granulosus* are infective immediately upon being passed in the faeces, and can remain infective in the environment for extended periods of time (Robertson and Thompson, 2002). Ingestion of infective eggs by humans leads to the development of hydatid cysts in the internal organs (commonly liver and lungs), impairing their function resulting in serious health problems (Thompson, 1986). Human infections are known to occur annually in Australia with treatment requiring major surgery, potentially combined with chemotherapy (Jenkins and Power, 1996). Absence of treatment may lead to mortality. The high prevalence of the pathogen *E. granulosus* within the peri-urban wild dog population, and the known movements and habits of peri-urban wild dogs indicates the potential risk to public health should be greatly considered.

Using post-mortem examination, hookworms appeared to be relatively low in prevalence (5.5%). Upon further testing utilising faecal float and molecular methods the proportion of samples positive to hookworms within the peri-urban wild dog population sampled markedly increased (Faecal float:17.2%, Molecular:39%). It is likely that the adult worms were not captured during the fine sieving process and hence were not detected. Ultimately, the prevalence of hookworms remained much lower than the 70.7% previously detected in scats from Far North Queensland (FNQ) (Smout et al., 2013) and 74% previously detected in 26 intestinal tracts in Townsville (Brown and Copeman, 2003). Our results were also less than the 37% prevalence in south-east Queensland reported by Jenkins et al. (2008). In contrast to *E. granulosus* climatic conditions such as increased humidity and temperature favour the presence of *Ancylostoma* spp.. The reason for the low prevalence of hookworm presented in these results could be attributed to the ability of *A. caninum*, the most commonly detected species, to undergo hypobiosis during the colder months (Gibbs, 1982). These differences could potentially explain the lower prevalence reported. *A. caninum* was unsurprisingly the most common species of hookworm detected. Hookworms are common in the domestic dog population in Australia (Palmer et al., 2008) with the majority of these being *A. caninum* (Palmer et al., 2007). Similarly within the wild dog population, *A. caninum* represents the main species detected amongst wild dogs (Brown and Copeman, 2003; Jenkins et al., 2008; Smout et al., 2013). Although detected in wild dog populations in FNQ (Smout et al., 2013), *A. ceylanicum* was not identified within our study area. Four wild dogs were found to have co-infections of *A. caninum* and *Uncinaria stenocephala* which has not previously been reported as far north as the greater Brisbane area. *U. stenocephala* is generally associated with the southern regions of Australia where lower temperatures, and cold winters are present (Palmer et al., 2007). *U. stenocephala*-positive dogs from our study were all trapped either in the month of May or August. The average minimum and maximum temperatures for May were 13.9 °C and 24 °C and for August were 9.8 °C and 23.3 °C respectively (Brisbane: www.bom.gov.au). The ideal temperature for larval development of *U. stenocephala* is 20 °C but can range from 7.5 °C up to 27 °C (Gibbs and Gibbs, 1959) well within the average temperature range across our study area.

Only two species of *Taenia* were detected throughout this study. The level of peri-urbanisation may limit the exposure of wild dogs to pathogens that are more frequent in livestock species over wildlife. *T. serialis* and *T. pisiformis* are commonly found in their intermediate stage in rabbits, and are probably more likely to be detected amongst the peri-urban wild dog population than *T. hydatigena* and *T. ovis* which use cattle, sheep and goats as intermediate hosts. Humans are accidental intermediate hosts for both species of *Taenia* detected in this study although records of cases are very rare (Ing et al., 1998). Therefore, the presence of *Taenia* species within the peri-

urban wild dog population would not appear to be of concern to public health or be of major economic significance for livestock industries.

Spirometra erinacei can be passed to humans via the consumption of pleuroceroids in the muscle of feral pigs (or other intermediate hosts) or through consumption of proceroids in untreated water (Lee et al., 1990b). The pathogen has been found to be highly prevalent (up to 80%) in northern Australian pig populations (Pavlov et al., 1992). Wild boar meat is commonly consumed in aboriginal communities around Australia (Koichi et al., 2012), although the local commercial market is small and hence consumption by the general Australian public is generally very low. The human health hazard is also reportedly low if meat is adequately frozen or cooked (Choquenot et al., 1996). Most wild boar meat is exported to Europe, with exports between 2006 and 2007 responsible for approximately AUD \$13 million contributed into the economy (Bengsen et al., 2014). The numbers exported each year, however, are highly volatile and largely depend on the demand (Gentle and Pople, 2013). Between 2001 and 2011 the number of feral pigs exported ranged between 73,000 and 322,000 with approximately 87% of these thought to originate from Queensland (AQIS, unpublished data). The results from this data suggest the high prevalence of *S. erinacei* in the peri-urban wild dog population could reflect prevalence in pig populations, which would potentially impact on the wild boar export trade through condemnations of infected carcasses. It could also be expected that there would be some risk for human infection from infected waterways. However, people in most peri-urban regions have access to treated drinking water and/or carry bottled water when spending time outdoors.

Toxocara canis was found at low levels within the intestines of the peri-urban wild dogs. These results are similar to previous work on the Sunshine Coast where only 4.6% of wild dogs were found to carry the roundworm (Jenkins et al., 2008). However, this is less than the prevalence detected in foxes in Western Australia where 14.9% were infected (Dybing et al., 2013). Geographic and demographic risk factors of both human (Congdon and Lloyd, 2011) and canine (Regis et al., 2011) toxocarosis are well described, however spatial factors and environmental distribution of infective eggs are not as well understood. Although young dogs in Bristol were found to shed the highest number of eggs, their movements were limited and hence contribution to environmental contamination was primarily through fox faeces (Morgan et al., 2013). Within the current study area, foxes (n=102) were found to have a moderately higher prevalence of *T. canis* than the wild dogs at 8.8% (see Chapter 4). The low prevalence in the wild dog population may suggest a lack of infective eggs in the environment (due to an increase in domestic dog anthelmintic use) and/or a lack of paratenic hosts in the diet that could contribute to infection in dogs and/or foxes. There is also the potential for age related immunity to be present and hence most infections in dogs above six months old could be somatic. Despite this, the potential impacts on human health

are significant and as such the presence of this pathogen within the peri-urban wild dog population should be noted. Although outside the scope of this study, future research should identify the presence of infectious eggs within the environment to gather more broad scale knowledge of the presence and/or the potential impact of this pathogen in peri-urban regions.

The variety of parasitic species co-detected in sampled dogs has been less than similar studies and unlike previously reported there were no unusual or unexpected parasites detected (Dybing et al., 2013; Jenkins et al., 2008). Peri-urban wild dogs across SEQ are mostly exposed to similar dietary items and exhibit similar preferences (Harriott, L. unpublished data), and items are usually readily available. Abundant and available food items may reduce the need to explore outside of their home range, and consume additional or 'non-traditional' food resources. Hence the exposure to different environmental sources of pathogens is probably limited. There were also discrepancies between the testing methods, suggesting identification of pathogens, particularly parasites, are often best identified by locating the adult worm. However molecular and faecal float methods were more sensitive in the detection of hookworms than through identifying adults within the intestines. Hookworms are small and fragile, and the freeze-thaw process may have impacted on the ability to locate the adults at processing. Recently there have been reports of the first findings of the tongue worm *Linguatula serrata* within the nasal cavities of wild dogs from rural areas in Australia (Jenkins, 2016). This was not detected in any of our necropsied cadavers. Similarly (Jenkins et al., 2014a; Smout et al., 2016) reported a high prevalence of heartworm (*Dirofilaria immitis*) in dingoes in FNQ. None of the 40 peri-urban wild dogs necropsied in our study were detected to have the presence of *Dirofilaria* within their heart or lungs.

The results indicate that carriage of potentially zoonotic bacterial pathogens is uncommon. As *E. coli* is a common bacterium than can naturally be found in the gastrointestinal tract of all animals and are frequently commensal rather than pathogenic organisms, this project was specifically interested in dogs that carried multi-drug resistant (MDR) *E.coli*. In the zones north, south and west of Brisbane there was at least one dog found to be carrying MDR *E.coli* although the area of greater Brisbane did not reveal any positive samples. Multiple samples were resistant to two or more drugs, and although the sample size was relatively small it opens the possibility for future research into this topic.

Salmonella Potsdam outbreaks in humans have been recorded from food contaminants relating to egg products. *Salmonella* Birkenhead is one of the most common causes of salmonellosis in humans in southern Queensland with 154 cases reported in 2006 (Stafford and Bell, 2006). Similar to our results, the majority of cases tend to occur south of Brisbane and cases tend to be sporadic infections rather than outbreaks. Researchers were not able to identify any risk factors of sporadic *Salmonella* Birkenhead infections in humans (Beard et al., 2004), and sources of environmental

contamination and wildlife populations appear to be insignificant. It is likely that *Salmonella* infections are random and opportunistic in peri-urban wild dogs. It is possible that their carriage of potentially pathogenic *Salmonella* serovars reflects environmental sources via predator-prey interactions. Their contribution to environmental contamination requires further exploration.

There were no *Campylobacter spp.* detected in the faecal material of wild dogs. This pathogen is difficult to culture and is very sensitive to freezing and other environmental stressors. As a result, we would not claim the bacteria is absent from the wild dog population, merely it was not detected in this study. Other pathogens that were not detected throughout the duration of the study include *Brucella suis* and *Rickettsia felis*. *Brucella suis* has recently been detected in pig-hunting dogs in north west NSW and QLD (Johnston, 2016) and further anecdotal case reports indicate it is present in SEQ dogs too. As a result, it is likely wild dogs can also harbour the disease. The capacity for wild dogs to act as long-term carriers of *Brucella suis* and as potential infectious sources for humans or other animals is unknown but requires investigation. Although the prevalence of *B. suis* in feral pigs in central Queensland has been known to be relatively high (Cook and Kingston, 1988) it is unknown if this pathogen has spread into the peri-urban environment. *Rickettsia spp.* was tested from whole blood samples as soon as feasibly possible. The DNA of the pathogen in blood can degrade quickly and extended storage time, repeated freezing and thawing (Cushwa and Medrano, 1993) can reduce ability to extract high quality and amplifiable DNA.

The results of the study should be interpreted in light of the limitations. First, the collection of wild dogs has been provided through a convenience sample where trapping was influenced by public complaints and/or existing wild dog management programs. Second, the requirement to manage the collection of samples in the field, particularly blood samples meant that they could not always be refrigerated instantly or tested within the ideal time frame. Similarly, we expect there to be some limitations in the bacterial results due to the difficulty of obtaining, storing, and testing samples within an appropriate amount of time. Finally, temporal aspects of epidemiology are not accounted for here, however, it is worth noting that this is the most comprehensive study of pathogens in peri-urban wild dogs, both in terms of sample size and number of pathogens screened, conducted to date in Australia. Our results have provided an extremely useful insight into the presence of pathogens in peri-urban wild dog populations in north-eastern Australia. Such information is essential to identify, inform and refine further, more detailed studies into significant pathogens. The data is also inherently important to as baseline data to determine whether presence of such pathogens is increasing, decreasing or being maintained into the future, essential to inform the strategic management of wild dog impacts in these areas (Fleming et al., 2014). However, more research is needed to determine the risk factors of individual pathogens and there is further opportunity to

conduct environmental screening for these same pathogens within public areas utilised by peri-urban wild dogs.

CONCLUSION

This is the most comprehensive study of pathogens in peri-urban wild dogs in Australia conducted to date. Our study demonstrates that parasitic pathogens of potential public health or economic impact are commonly found within the intestinal tracts of peri-urban wild dogs, most notably *E. granulosus* and *A. caninum*. Future research in wild dogs should focus around these two pathogens and monitoring of livestock at abattoirs, wildlife and humans for hydatid cysts would be of value. The high presence of *S. erinacei* should be also noted given the importance of the wild boar export trade but it is unlikely to impact public health directly in Australia. Finally our study indicates that peri-urban wild dogs do not seem to be carriers of significant bacterial species. However, limitations in the sampling and testing methods used in the current study indicate that there is scope to conduct further research into these pathogens, particularly in the field of multi-drug resistant bacteria.

CHAPTER 4

Diet of peri-urban wild dogs and its association with
zoonotic pathogen carriage

ABSTRACT

In Chapter 3 we identified the zoonotic pathogens harboured by peri-urban wild dogs. Some of these pathogens are transmitted to wild dogs through consumption of prey. In this chapter we present an analysis on wild dog dietary composition to evaluate the potential influences of food selection on zoonotic pathogen carriage rates. Furthermore, understanding the role of diet and resource use can add to the knowledge of wild dog ecology and assist with the implementation of management strategies for the wild dog population. Information on wild dog diet relies mostly on data collected from scats, which may hinder the detection of particular food sources. Here we report the diet composition from 170 peri-urban wild dogs by analysing the content of stomachs. The majority of food items detected in the stomach were mammalian prey species, most commonly: swamp wallaby ($20.6 \pm 6.08\%$); canine species (prey) ($10.6 \pm 4.62\%$); eastern grey kangaroos ($10.0 \pm 4.51\%$); and deer species ($10.0 \pm 4.51\%$). The presence of unidentified bird species ($10.0 \pm 4.51\%$) was also one of the most commonly detected items. Wild dogs that consumed swamp wallaby were found to be significantly more likely to be infected with *Echinococcus granulosus*. This finding demonstrates the importance of managing both the definitive and intermediate stages of the tapeworm. Additional environmental risk factors pertaining to *E. granulosus* infection in peri-urban wild dogs should be addressed in future research. Addressing environmental risk factors will allow for further understanding of potential disease drivers and may lead to improved strategies to manage pathogens in wild dogs which are currently unmanaged.

INTRODUCTION

Many wildlife species worldwide have experienced increasing pressure to adapt to urbanisation (Ditchkoff et al., 2006). Species that can adjust and be flexible with their resource requirements generally maintain successful populations within the peri-urban or urban environment (Romig et al., 2015). In Australia, as a result of interbreeding between the largest terrestrial predator, dingoes (*Canis lupus dingo*) and domestic dogs (*Canis familiaris*), a wild dog population of hybrid animals is now common across the mainland (Stephens, 2011). Wild dogs have adjusted well to the peri-urban environment and as a result can be found in the majority of locations across the country, particularly across the eastern seaboard where the human population is significantly increasing (Allen et al., 2013). Human conflict with wild dogs generally relates to predation or harassment of hobby farm livestock, backyard poultry, domestic pets, as well as the impact on conservation of our native wildlife and potential impacts on human health (Allen et al., 2016; Jenkins et al., 2008). Dietary information can address concerns regarding predation on locally threatened native wildlife, and provide ecological information about wild dogs which is beneficial for general knowledge but can also advance management strategies to alleviate human-wildlife conflicts (Murray et al., 2015). Current dietary information for wild dogs relies mostly on scat collections and is typically reported from wild dog populations in rural areas (Brook and Kutt, 2011; Corbett, 2001b). Wild dogs are known to predate large (Cupples et al., 2011; Whitehouse, 1977), medium (Claridge et al., 2010) and small sized mammals (Corbett, 2001b) depending on which species are common and abundant locally (Brook and Kutt, 2011; Claridge et al., 2010). Allen et al. (2016) found small to medium sized mammals are the most frequent prey item found within scats of peri-urban wild dogs from north-eastern Australia. While scat data is easy to access and allows repeated consistent sampling across different habitats, there are some limitations which only stomach sampling can overcome (Balestrieri et al., 2011). Collection of stomachs allows for more detailed analysis (i.e. reporting of biomass) and provides accurate detection and identification of items that may go unreported from scat studies, especially feathers from bird species (Cavallini and Volpi, 1995). If peri-urban wild dogs are consuming significant amounts of human associated food items (e.g. bread, domestic dog food), these may be masked from scat studies and therefore give a biased representation of diet composition (Balestrieri et al., 2011). Understanding food and dietary preferences of peri-urban wild dogs adds to our general knowledge of these predators, assists pest management programs to develop strategies and apply adaptive programs to manage human-wildlife conflicts and conservation efforts of native species (Allen and Leung, 2012).

A number of studies have demonstrated the influence of diet on wildlife host-pathogen interactions (Cross et al., 2007; Hegglin et al., 2007; Jessop et al., 2012; Miller et al., 2003). However, understanding whether diet affects the presence of pathogens in wild dogs has not been

investigated, nor discussed. Our research indicates that peri-urban wild-dogs can carry pathogens of public health significance (see Chapter 3). Dogs act as definitive hosts for a variety of parasites whose lifecycles are perpetuated by the ingestion of parasite stages in intermediate paratenic hosts that are naturally preferred prey species (Jenkins and MacPherson, 2003). Hence, the prey–predator relationship is consequently a key factor in understanding the transmission dynamics of infectious pathogens. For example, macropod species are known to be a preferential food resource for wild dogs (Allen et al., 2016). They are also known to be highly suitable intermediate hosts to the hydatid tapeworm (Jenkins and Morris, 2003). It is therefore likely that there is a link between dietary preference of an individual wild dog and its potential to become infected with a zoonotic pathogen. Previous research suggests that wild dogs are largely reliant on just one or two species of primary prey in any given location, with scat data from the same location in 2002 and then again in 2013 showing an almost identical degree of dietary overlap (Allen et al., 2016). Hence, a relatively stable dietary preference of a wild dog may indicate its potential to be infected with a pathogen.

This study aims to investigate the diet composition of peri-urban wild dogs in south-east Queensland and north eastern New South Wales and its association with parasitic and bacterial infection status of each wild dog. More comprehensive dietary studies based on stomach contents, rather than scats, will fulfil gaps in our knowledge where particular food items may not have been detected. Understanding the association between diet composition and the presence of infectious pathogens will allow us to determine the role that prey species have in the maintenance of infected wild dog populations. This information could be critical to future wild dog management programs, especially where colonisation of wild dogs in peri-urban and urban regions may increase infection pressure to humans in densely populated areas. Therefore knowledge about transmission factors is directly relevant to public health.

METHODS

Study area

The study area was described previously in Chapter 3. In brief, the study area was divided into four regions: Greater Brisbane (GB); North Brisbane (NB); West Brisbane (WB); and South Brisbane (SB). Each wild dog was allocated to one of the four regions based on capture location. The number of dogs from each region is indicated in Table 4.1. Wild dog carcasses for study were supplied through council or private pest management programs undertaken within the study area between August 2012 and May 2015. All wild dogs were culled as part of routine pest management programs, and the supply of carcasses approved for necropsy by the University of Queensland Animal ethics committee (approval number SVS/145/13).

Collection of stomachs and diet composition

Dogs were sampled as part of a broader survey strategy (see Chapter 3). Whole stomachs (n=170) were removed at necropsy, individually bagged and stored frozen at -20 °C. Stomach contents were washed, air-dried and stored in paper bags before being sent for dietary composition analysis by a professional service provider (Barbara Triggs). Identification of contents was primarily based on morphological characteristics of mammalian hairs. Other items such as bones, feathers, invertebrates and anthropogenic items were recorded. All food items were identified to the lowest taxonomic level possible, and the mass (g) and volume (%) recorded. Stomachs were collected after the some of the initial necropsies had occurred. Hence, the sample size of stomachs is slightly lower than the total number of wild dogs sampled.

Pathogen Identification

Parasitic and bacterial pathogens were identified in wild dogs following necropsy, utilising adult worm identification, faecal floatation and egg identification, and molecular or microbiological methods described previously. These results can be found in detail in Chapter 3. In brief, parasites were the most commonly detected pathogens with: *Echinococcus granulosus* adult worms detected within 50.7 ± 6.9 % of intestines, followed by *Spirometra erinacei* (36.6 ± 6.4 %); Hookworms, including *Ancylostoma caninum* and *Uncinaria stenocephala* (28.8 ± 7.1 %); *Toxocara canis* (5.4 ± 3.1 %) and *Taenia spp* including *T. serialis* and *T. pisiformis* (4.5 ± 2.8 %). Only 18 (8.95%) of peri-urban wild dogs were found to have a positive bacterial isolation from faeces - two dogs were infected with two species of bacteria. Bacterial pathogens detected included *Escherichia coli* (20 ± 10.1 %), *Salmonella spp* (3.7 ± 3.7 %) and methicillin sensitive *Staphylococcus aureus* (3.3 ± 2.7 %).

Data analyses

To assess the adequacy of the sample size, we adopted the method used by Gentle et al. (2015). Wild dog stomachs were randomly batched into groups of five. Their relationship with the overall sample size was compared utilising the cumulative number of prey items and the Brillouin Index (H_B). The diversity of the diet was calculated using:

$$H_B = \frac{\ln N! - \sum \ln n_i!}{N}$$

where N is the total number of individuals in the sample and n_i is the number of individuals in the i th species. The Brillouin index is suggested for non-random sampling (Pielou, 1975).

For each dietary item identified the frequency of occurrence and biomass of stomach contents was estimated. Frequency of occurrence of a particular food item was estimated as a proportion by

dividing the number of stomachs containing each food item by the total number of stomachs analysed. Biomass was estimated as a proportion by dividing the weight of each food item per stomach by the total weight of the stomach. Univariable logistic regression was used to determine if dietary composition and biomass changed across the seasons. Initially, the four seasons (winter, spring, summer, autumn) were analysed, however results from this lead to a reduction of seasonal groupings to dry (May – October) and wet (November – April) seasons.

To determine whether dietary composition was associated with infection status of each wild dog, we compared the presence of the main prey species consumed with the presence and absence of pathogens of interest. For the purpose of the analysis we considered parasitic pathogens that had high prevalence (as reported in Chapter 3), represented potential risk to human health, and involved an intermediate host where ingestion can be a transmission method. Based on these terms the pathogens of interest were: *Echinococcus granulosus*; *Spirometra erinacei* and hookworms (including *Ancylostoma caninum* and *Uncinaria stenocephala*). Feeding behaviour of wild dogs is likely to be affected not only by spatial factors, but also by individual factors (e.g. age and sex). Firstly, univariable logistic regression models (Family, Bernoulli) were constructed including *E. granulosus* infection as the response variable and individual factors (age and sex) and diet categories as univariable predictors. Due to the knowledge of macropod species as intermediate hosts for *E. granulosus* the four main macropod species (see table 4.3) were analysed against non-macropod species as the reference category. Variables significant at a $p < 0.20$ were then considered for a full multivariable model. Secondly, a final multivariable logistic regression model accounting for animal age, sex and a random effect for region was determined by backwards stepwise regression. The same analytic approach was conducted for other pathogens of interest (Hookworm and *S. erinacei*). However, because of lower prevalence of infection for these pathogens, dietary items were classified into more general categories including: macropods; canine; bandicoot; other mammals; vegetation; and birds. *S. erinacei* has an important aquatic stage in its lifecycle. To determine if distance to natural water sites influenced infection we measured from point of capture to the closest natural water site utilising geographical information systems (GIS) and included this data in the logistic regression. All statistical analyses were conducted in STATA/IC 13.1 (StataCorp. 2013. *Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP) and GIS work conducted in ArcGIS (ESRI, ArcGIS Desktop 10.3).

Wild dogs that exhibited bacterial infections of any kind were also compared with their corresponding dietary items. The small sample size of wild dogs with bacterial infections precluded formal statistical analyses to be conducted and hence descriptive statistics were used.

RESULTS

The sample size used for this study was sufficient for describing the diversity of peri-urban wild dog dietary habits. Brillouin's Index reached an asymptote at approximately 70 stomachs and no new food groups were recorded after sampling of 50 stomachs, confirming that the sample size (170 stomachs) exceeded that required to sufficiently describe diet.

Diet composition

A total of 170 peri-urban wild dog stomachs were collected and analysed for dietary composition (Table 4.1). Overall, the most common prey species identified was swamp wallaby (*Wallabia bicolor*), being present in 20.7% of peri-urban wild dogs. This was followed by canines (*Canis spp.* (prey)) and bird feathers, both present within 10.6%. Eastern grey kangaroo (*Macropus giganteus*) and unidentified deer species were both also present within 10.0% of stomachs. Mammalian prey species were absent in 15.9% of stomachs (i.e. no hair found). The vast majority (93.5%) of wild dog stomachs featured only one prey species. This is also represented by the high biomass percentage of most common prey items. The remaining 6.5% percent featured one additional (i.e. a second) prey species, but generally only trace amounts of the second prey species was detected. The presence of vegetation (grass and/or leaves) was also common, however, only accounted for trace or very low amounts within the stomach (71% contained <1 g). Eleven stomachs were recorded where grass was the sole content item. Most contained only trace amounts (< 2 g) but one containing 58 g of vegetative matter. There was no detection of the mesopredators red fox (*Vulpes vulpes*) or feral cat (*Felis catus*) in any of the samples. Human associated food items (commonly food product wrappers) were detected in 7% of stomachs.

The occurrence and biomass of the eight most common overall dietary items were calculated (Table 4.2). Most of the major dietary items were present in both the wet and dry seasons. The occurrence of deer within stomach contents during the dry season was noticeably less than in the wet season. However, the occurrence of northern brown bandicoot was noticeably higher in the dry season than the wet. Vegetation occurred the most frequently in both seasons however it presented the least biomass.

Table 4.1 Comparison of the proportion of wild dog stomachs in which various food items were detected from the four regions within the study area including the overall occurrence and overall biomass for each dietary item.

Type	Common Name	Scientific Name	Greater Brisbane (n=49) %	North Brisbane (n=39) %	South Brisbane (n=51) %	West Brisbane (n=31) %	Overall occurrence (n=170) %	Overall biomass %
Mammals	Antechinus sp.	<i>Antechinus sp.</i>	2.04	0.00	0.00	0.00	0.58	Trace
	Australian Swamp Rat	<i>Rattus lutreolus</i>	2.04	0.00	0.00	0.00	0.58	80.00
	Black Rat	<i>Rattus rattus</i>	0.00	2.56	0.00	0.00	0.58	97.62
	Cattle	<i>Bos taurus</i>	6.12	7.69	0.00	0.00	3.53	97.01
	Common Ringtail Possum	<i>Petrus peregrinus</i>	0.00	0.00	0.00	3.22	0.58	85.00
	Deer	various.	22.44	5.12	5.88	3.22	10.00	90.55
	Dingo/Dog (grooming)	<i>Canis sp. (grooming)</i>	6.12	2.56	0.00	6.45	3.53	Trace
	Dingo/Dog (prey)	<i>Canis sp. (prey)</i>	12.24	12.82	1.96	19.35	10.60	87.21
	Eastern Grey Kangaroo	<i>Macropus giganteus</i>	8.16	20.51	1.96	12.90	10.00	82.50
	European Rabbit	<i>Oryctolagus cuniculus</i>	0.00	5.12	1.96	3.23	2.35	90.91
	Feral Pig	<i>Sus scrofa</i>	4.08	0.00	0.00	0.00	1.18	22.50
	Greater Glider	<i>Petauroides volans</i>	0.00	0.00	0.00	3.23	0.58	73.08
	Long Nosed Bandicoot	<i>Perameles nasuta</i>	0.00	0.00	1.96	0.00	0.58	100.00
	Northern Brown Bandicoot	<i>Isodon macrourus</i>	8.16	5.12	7.84	12.90	8.23	91.95
	Possum species	<i>Trichosurus sp.</i>	0.00	2.56	3.92	3.23	2.35	86.46
	Red Necked Wallaby	<i>Macropus rufogriseus</i>	4.08	0.00	1.96	0.00	1.76	74.98
	Sheep	<i>Ovis aries</i>	2.04	5.12	0.00	0.00	1.76	69.70
	Squirrel Glider	<i>Petaurus norfolcensis</i>	2.04	0.00	0.00	0.00	0.58	14.45
	Swamp Wallaby	<i>Wallabia bicolor</i>	14.29	10.26	41.18	6.68	20.58	89.62
	Unknown Macropus species	<i>Macropus spp.</i>	0.00	0.00	0.00	19.35	3.53	99.22
No Hairs	No Hairs		18.37	7.69	23.52	9.68	15.88	-
Other	Beetle		2.04	0.000	1.96	0.00	1.18	100.00
	Birds		12.24	10.26	11.76	6.45	10.60	85.82
	Bone fragments		2.04	12.82	9.80	3.23	0.070	90.91
	Eggshell		2.04	0.00	1.96	0.00	1.18	45.04
	Emasculation Ring		2.04	0.00	0.00	0.00	0.58	Trace
	Fish bones/scales		2.04	0.00	0.00	0.00	0.58	100.00
	Human Hair		0.00	0.00	0.00	3.23	0.58	Trace
	Net/Mesh/Wire/String		6.12	5.12	0.00	0.00	2.35	5.34
	Paper		6.12	7.69	11.76	3.23	7.06	5.54
	Reptile		0.00	5.12	0.00	0.00	1.18	34.09
	Sponge		0.00	5.12	1.96	6.45	2.94	2.83
	Stones		0.00	0.000	1.96	3.23	1.18	60.94
	Vegetation		67.35	69.23	74.51	74.19	71.18	17.89

Table 4.2 Comparison of the major dietary items of peri-urban wild dogs showcasing a) occurrence (%) and b) biomass (%) across the wet and dry seasons

Occurrence (%)	(n=93)	(n=71)
	Wet	Dry
Birds	18.31 ± 7.05	5.63 ± 5.36
Deer	16.90 ± 6.81	2.82 ± 3.84
Dingo/Dog	18.31 ± 7.05	14.08 ± 8.09
No Hairs	22.54 ± 7.67	14.08 ± 8.09
Northern Brown Bandicoot	4.23 ± 3.59	11.27 ± 7.35
Other macropods	16.90 ± 6.81	16.90 ± 8.71
Swamp wallaby	26.76 ± 8.19	19.72 ± 9.25
Vegetation	90.14 ± 9.42	76.06 ± 9.92
Biomass (%)		
	Wet	Dry
Birds	76.45 ± 9.87	71.61 ± 9.86
Deer	91.67 ± 6.42	81.98 ± 6.42
Dingo/Dog	66.36 ± 10.99	62.01 ± 9.86
No Hairs	0.00	0.00
Northern Brown Bandicoot	89.2 ± 7.22	99.26 ± 1.74
Other macropods	87.88 ± 7.59	93.05 ± 5.16
Swamp wallaby	81.28 ± 9.07	81.17 ± 7.94
Vegetation	37.02 ± 11.23	18.6 ± 7.91

Relationship between Echinococcus granulosus and diet

Within the population of wild dogs in our study area, 13.02% ($\pm 5.05\%$) of sampled animals had both the presence of *E. granulosus* in their intestines and swamp wallaby in their stomach. Data for the other species of macropods and non-infected animals can be found in Table 4.3. After accounting for age, sex and clustering of samples by region (Table 4.4), dogs that consumed swamp wallaby (Diet 1) were 1.79 times more likely to have an *E. granulosus* infection ($p < 0.05$) compared to dogs that did not consume a macropod species. Similarly, wild dogs that consumed unidentified macropod species (Diet 4) were 4.18 times more likely to have an *E. granulosus* infection ($p < 0.01$). The unidentified *Macropus* species were suspected as *M. robustus* ($n=3$), *M. dorsalis* ($n=2$) and *M. rufogriseus* ($n=1$).

Table 4.3 Percentage of species of macropods found in the stomachs of peri-urban wild dogs, with those positive for *Echinococcus granulosus* and those negative. Average worm burdens are shown for *Echinococcus* positive dogs.

	<i>Echinococcus</i> +ve (n=88) N (%)	<i>Echinococcus</i> -ve (n=81) N (%)	Average worm burden
<i>M. giganteus</i>	7 (4.14 \pm 2.99)	10 (5.92 \pm 3.54)	9318
<i>M. rufogriseus</i>	1 (0.59 \pm 1.15)	2 (1.18 \pm 1.62)	140
<i>W.bicolor</i>	22 (13.02 \pm 5.05)	12 (7.10 \pm 3.86)	12772
<i>Macropus</i> sp.	4 (2.37 \pm 2.28)	2 (1.18 \pm 1.62)	35527

Table 4.4 Univariable and multivariable analyses of age, sex and diet on intestinal *Echinococcus granulosus* infection in peri-urban wild dogs with non-macropod species as the reference category.

Variables	Univariable			Multivariable		
	Odds Ratio	95% Confidence interval	P-value	Odds Ratio	95% Confidence interval	P-Value
Sex(Female)	0.70	0.46 - 1.07	0.104	0.72	0.48 - 1.08	0.109
Age (6-12 months)	0.67	0.34 - 1.35	0.266	0.68	0.35 - 1.28	0.232
Age (1-2 years)	1.01	0.39 - 2.63	0.988	0.87	0.35 - 2.16	0.771
Age (>2 years)	1.51	0.65 - 3.50	0.338	1.06	0.50 - 2.25	0.866
Age (Overall)			0.697			
Diet 1 (W. bicolor)	1.74	1.02 - 2.99	0.003	1.79	1.12 - 2.85	0.015
Diet 2 (M. giganteus)	0.67	0.17 - 2.61	0.562			
Diet 3 (M. rufogriseus)	0.89	0.06 - 13.26	0.934			
Diet 4 (Macropus spp.)	3.70	1.64 - 8.37	0.002	4.18	1.80 - 9.74	0.001

Relationship between Hookworm and diet

Accounting for age, sex and clustering of samples by region (Table 4.5), peri-urban wild dogs that had consumed northern brown bandicoots (*Isodon macrourus*) and unknown bird species were respectively 3.09 and 7.80 times more likely to have hookworm infections than those that consumed other dietary resources (Macropods). There also appears to be significance amongst the age variables however this will be examined in detail in Chapter 6.

Table 4.5 Univariable and multivariable analyses of age, sex and diet on hookworm infection in peri-urban wild dogs with macropods as the reference category.

Variables	Univariable			Multivariable		
	Odds Ratio	95% Confidence interval	P-value	Odds Ratio	95% Confidence interval	P-Value
Sex(Female)	1.00	0.25 – 3.95	1.000	1.19	0.19 - 7.28	0.850
Age (6-12 months)	0.61	0.27 - 1.37	0.230	0.47	0.25 - 0.89	0.020
Age (1-2 years)	2.03	1.21 – 3.38	0.007	2.00	0.60 - 6.67	0.261
Age (2-5 years)	0.65	0.23 - 1.85	0.421	0.38	0.07 - 1.98	0.249
Age (Overall)			0.000			
Diet 1 (N B bandicoot)	1.87	0.77 – 4.53	0.164	3.09	1.43 - 6.70	0.004
Diet 2 (Canis)	1.79	0.86 – 3.74	0.220			
Diet 3 (Other mammals)	0.89	0.54 - 1.49	0.673			
Diet 4 (Vegetation)	0.85	0.73 – 0.99	0.466			
Diet 5 (Bird)	5.63	0.99 - 32.22	0.052	7.80	5.42 - 11.21	0.000

Relationship between S. erinacei and diet

Accounting for age, sex and clustering of region (Table 4.6) peri-urban wild dogs that had consumed vegetation in preference to other mammalian species (Macropods) were 2.37 times more likely to be infected with *Spirometra erinacei*. There was no difference in average distance to natural waterways (creeks) for *S. erinacei* infected dogs with and without vegetation in their stomachs as well for the non-infected dogs.

Table 4.6 Univariable and multivariable analyses of age, sex and diet on *Spirometra erinacei* infection in peri-urban wild dogs with macropods, males and dogs under 6 months as the reference categories.

Variables	Univariable			Multivariable		
	Odds Ratio	95% Confidence interval	P-value	Odds Ratio	95% Confidence interval	P-Value
Sex(Female)	0.74	0.33 – 1.68	0.479	0.87	0.35 – 2.19	0.782
Age (6-12 months)	0.57	0.39 – 0.84	0.124	1.02	0.39 – 2.68	0.957
Age (1-2 years)	2.10	1.31 – 3.34	0.059	2.71	0.83 – 8.87	0.100
Age (>2 years)	1.40	0.89 – 2.21	0.148	1.98	0.79 – 4.99	0.145
Age (Overall)						
Diet 1 (N B bandicoot)	0.88	0.33 – 2.34	0.801			
Diet 2 (Canis)	0.75	0.49 – 1.12	0.155	0.72	0.38 – 1.29	0.268
Diet 3 (Other mammals)	0.85	0.32 – 2.21	0.737			
Diet 4 (Vegetation)	2.18	1.39 – 3.40	0.001	2.37	1.62 – 3.46	0.000
Diet 5 (Bird)	1.13	0.46 – 2.75	0.788			
Dist to Water	1.04	0.69 – 1.56	0.861			

Bacterial pathogens and diet

A greater percentage (82.4%) of wild dogs with targeted bacterial pathogens present in stomach contents had consumed vegetative material than dogs without bacterial infections (70.6%), but only trace and/or low amounts of vegetation were recorded. Predatory dietary items appeared to be random; with no common themes (Table 4.7). Formal analyses were not completed given sample numbers are low, hence data is descriptive.

Table 4.7 Bacterial pathogens present in peri-urban wild dogs including their dietary items

Bacterial Infection	Predatory dietary item	Vegetation
<i>Salmonella spp.</i> (n=3)	Deer (n=1) Dingo/Dog (prey) (n=1) No diet data (n=1)	Vegetation (n=1)
<i>Staphylococcus aureus</i> (n=5)	No hairs (n=1) Swamp wallaby (n=2) Northern brown bandicoot (n=2)	Vegetation (n=4)
AMR <i>Escherichia coli</i> (n=12)	Dingo/Dog (prey) (n=1) No hairs (n=3) Northern brown bandicoot (n=2) Mammal bones (n=3) Swamp wallaby (n=1) Greater glider (n=1) Dingo/Dog (grooming) (n=1)	Vegetation (n=11)

DISCUSSION

The findings of this research chapter are unique in that they describe the diet of peri-urban wild dogs in Queensland using dietary data collected from stomach contents. This study is also the first to assess the associations between diet composition and the presence of predominant zoonotic pathogens in peri-urban wild dogs in Australia. Our results confirm that peri-urban wild dogs depend predominately on mammalian prey, consistent with a recent scat analysis from peri-urban wild dogs in southern Queensland (Allen et al., 2016). Similar to the Allen et al. (2016) study both swamp wallaby (*W. bicolour*) and northern brown bandicoot (*I. macrourus*) were frequently represented in wild dog stomachs across SEQ. However, our study had a higher representation of dingo/wild dog (*Canis spp.*) and eastern grey kangaroo (*M. giganteus*) as prey, and a lower representation of birds. The high presence of deer consumed by peri-urban wild dogs in the greater Brisbane region corresponds with known feral populations of deer on various species (red, chital, sambar and fallow). Red deer were historically concentrated within the Brisbane Valley, although more recent introductions other species have established wild populations and expanded distribution (Stuart et al., 2013). Deer tissue was recorded in the stomach of wild dogs throughout peri-urban areas in south-eastern Queensland, as far north as the Valdora area on the Sunshine Coast and as far south as Maudsland on the Gold Coast, suggesting that deer are widely utilised as prey. Interestingly, this is in contrast to the recent dietary study utilising scat data which only found deer in the diet of dogs from the Brisbane Valley, with no traces from other regions of south-east

Queensland (Allen et al., 2016). Our data could be considered an accurate representation of the diet of peri-urban wild dogs given the relatively accurate detection and identification of food items from stomach analysis (Cavallini and Volpi, 1995). Hence, it is worth noting that stomach sampling has revealed that deer, an invasive herbivore, are a dietary resource for wild dogs throughout peri-urban south-eastern Queensland.

Peri-urban wild dogs are known to travel into suburban backyards (Allen et al., 2013) with several anecdotal reports suggesting they opportunistically consume domestic pet food. Domestic dog food was not detected within this study, suggesting that it is not a significant food resource. Across the globe, human associated food resources are highly utilised by urban vertebrate pests (Contesse et al., 2004; Newsome et al., 2015). Coyotes were found to utilise anthropogenic sources of food when they exhibited signs of illness, however healthy coyotes preferred to utilise natural sources of food (Murray et al., 2015). Dingoes in the Tanami desert often use human-provided rubbish as a food source (Glickman and Schantz, 1981; Lee et al., 1990b) however in peri-urban areas where waste disposal systems are relatively predator proof, the opportunity (and desire) for wild dogs to access sources of anthropogenic food may be reduced. Despite evidence of contact with these resources (e.g. paper, food product wrappers), such items do not make up a significant amount of dietary content. Our data further supports that of Allen et al. (2016) in suggesting that peri-urban wild dogs are not reliant on sources of anthropogenic food. The provision of anthropogenic food resources have been shown to have neutral, positive and negative effects on pathogen infection rates in wildlife (Becker et al., 2015). Rock Iguanas fed supplementary dietary items by tourists presented with increased hookworm infections (Knapp et al., 2013) however, alternatively, urban foxes within residential environments expressed better immune status to a number of canine viruses and were less likely to be infected than exurban foxes (Cypher and Frost, 1999). The lack of consumption of anthropogenic resources by peri-urban wild dogs presents a greater opportunity for predator-prey interactions and hence greater dietary exposure to pathogens.

The occurrence of the major dietary items appeared to vary across the seasons indicating that wild dogs consume different prey species during the wet season. In particular, the occurrence of birds and deer consumed increased drastically in the wet season comparison to other dietary resources. Bird consumption was also associated with the presence of hookworms. The lifecycle of hookworms exhibits seasonal variation where eggs shed in canine faeces develop in suitably moist and warm environments, conditions present during the wet season. *A. caninum*, the most common species of hookworm present in wild dogs across south-east Queensland (see. Chapter 3) undergo hypobiosis during the dry season, and adaptation to enhance survival of the parasite (Gibbs, 1982). This ensures maximum egg output coincides with the onset of the wet season when climatic conditions for larval development are ideal. The main transmission route of hookworms in canines

is through transmammary transmission (Stone and Girardeau, 1968). However, any mammal is capable of acting as a paratenic host to hookworm (Lee et al., 1975). Birds have been shown to be paratenic hosts of hookworm where larvae migratory patterns to the muscles and organs are similar to what is seen in mice (Agarwal and Agarwal, 1983; Agarwal and Johri, 1980). Patent infections developed in pups that had consumed infected chickens (Mittra and Sasmal, 1985) however larval yield from the infected chickens dramatically decreases 72 hours post infection resulting in a short time frame where canines could become infected (Agarwal and Johri, 1980). Hence, an increased biomass of birds during the wet season, when the climatic conditions for hookworm are ideal, could relate to an increased presence of hookworms in peri-urban wild dogs. Alternatively, birds may be secondarily associated with hookworm where they act as an indicator of risk within environments that provide ideal survival of hookworm larvae. More research is needed to investigate the potential role of small mammals and birds in the transmission route of hookworms to wild dogs.

Swamp wallaby was consumed by wild dogs in all four regions, and was overall the most prevalent prey item consumed. Wallabies have long been a staple diet for dingoes in Australia (Banks, 1984; Durie and Riek, 1952) and our data suggest that swamp wallabies are an important resource for peri-urban wild dogs. Along with other macropod species, they are known intermediate hosts of *Echinococcus granulosus* (Barnes, 2007). The high reliance on macropod species as a staple dietary item is hypothesised to be a significant factor in the high prevalence of *E. granulosus* within the wild dog population (Coman, 1972). Unlike dogs, where there are often no clinical signs of infection with adult *E. granulosus* tapeworms, macropods with hydatid cysts experience massive loss of lung volume and severe respiratory conditions, thus exposing them as easy prey for predators (Jenkins and MacPherson, 2003; Jenkins et al., 2005). As well as macropods, both cattle and sheep can be intermediate hosts to the hydatid tapeworm, but domestic stock is rarely recorded in peri-urban dog diets (Allen et al. 2016, current study). Hence, our results support the hypothesis that the sylvatic cycle has significant impacts on infection of peri-urban wild dogs with *E. granulosus* sourced through consumption of macropod species. After accounting for age, sex, and regional clustering, peri-urban wild dogs that had recently consumed swamp wallaby were significantly more likely to be positive with *E. granulosus*. However, it is important to note that there are numerous other factors responsible for *E. granulosus* presence within an area. Both physical and climatic environmental factors can have a large influence on the presence of *E. granulosus* eggs and their ability to survive within the environment and hence infect an intermediate host. These factors are analysed and discussed in depth in Chapter 5 in the context of environmental drivers of wild dog zoonotic pathogen infection.

The association between *Spirometra erinacei* infection and the presence of vegetation in the diet is unexpected due to the requirement of an aquatic cycle involving copepods as intermediate hosts

(Lee et al., 1990a). Infection in dogs requires consumption of an infected intermediate and / or subsequent paratenic host such as amphibians, reptiles, and feral pigs. The role of distance to water sources was not significant for vegetation consumption and infection, suggesting little role in transmission. However, wild dogs are known to travel extended distances as part of daily activities, including use of watering points, and thus that the distance to nearest water source may not adequately represent exposure to such environs. The limited public health significance of *S. erinacei* from wild dogs suggests limited need for further research into this topic.

Due to low sample size, the bacterial results are likely inconclusive to suggest that dietary items are influencing the presence of bacterial pathogens within wild dogs. Nevertheless, diet remains an obvious and likely influence on bacterial infections. Bacteria can be transmitted through environmental contamination, however, both dogs with and without bacterial infections had high prevalence, but little biomass, of vegetative matter within their stomachs. It is highly likely that bacterial infections in peri-urban wild dogs are sporadic, and diet may be the initial cause of infection, but a larger sample size of peri-urban wild dogs with bacterial infections is required to determine potential casual factors.

This study is the only recent data on peri-urban wild dog diets to utilise stomach analyses. This provides data on both occurrence and biomass that allows a comprehensive picture of dietary composition. In a novel approach, we have been able to combine this information with the status of wild dog pathogens to examine diet as a pathway to infection. It is important to acknowledge that, without multiple samplings of each individual, dietary studies can only provide a ‘snapshot’ of the diet at each sampling period. It is also unknown how long adult worms can remain in the intestines of wild dogs however in many cases they are thought to survive for extended amounts of time. This is important because we cannot determine when an animal became infected, and whether young, infected animals re-locate and introduce infective eggs into a previously uninfected environment. The abundance of prey may also differ across the seasons, influencing availability for capture and consumption by wild dogs. Regardless, the associations presented here, particularly between swamp wallaby consumption and *E. granulosus* infection are epidemiologically significant, and support hypotheses generated from previous studies, and warrant further detailed study.

Despite some limitations, our data is epidemiologically significant and suggest consumption as a key transmission pathway which needs to be considered in future studies. Densities of prey species, as well as knowledge of environmental sources of pathogens, are required to further elucidate linkages. It is likely that variation in both biomass and dietary composition between seasons is influenced by the availability and distribution of prey items in each region, rather than dietary preference. However without knowledge of seasonal fluctuations in prey densities and distribution, this cannot be confirmed. Research pertaining to peri-urban wildlife and peri-urban wild dogs in

particular remains critically important, and our study provides excellent baseline data which would be beneficial to guide future research.

CONCLUSION

Our data confirms that mammalian prey is the most predominant dietary resource in peri-urban wild dogs. Anthropogenic sources of food do not seem to have an important role in maintaining populations within the peri-urban regions, suggesting that reducing the availability of human associated food items or the presence of uneaten dog food is unlikely to impact on the presence of wild dogs within urban areas. There is a significant association between the consumption of swamp wallaby and the presence of *E. granulosus* infection in wild dogs. This is likely to assist with the maintenance of the high presence of the pathogen within the wild dog community and has the potential to have major implications on public health. Management strategies to reduce the presence of *E. granulosus* in both the adult form in wild dogs and the metacestode stage in swamp wallabies may need to be considered. The increased biomass of birds consumed during the wet season coincides with the lifecycle of hookworms, but more information regarding the role of birds as a prey species and their contribution to hookworm infection is required. Future studies should address additional risk factors pertaining to *E. granulosus* presence in particular environmental and climatic factors.

CHAPTER 5

Geographical distribution and risk factors for
Echinococcus granulosus carriage in peri-urban wild
dog populations

ABSTRACT

In Chapter 3, *Echinococcus granulosus* was identified as the predominant pathogen carried by peri-urban wild dogs. In Chapter 4, dietary selection was identified as potentially influencing the presence of *E. granulosus* within the wild dog population. Following this, further investigations were conducted into estimating the Index of Potential Contamination (IPC) and the role of the physical environment, climate and individual factors in determining the geographical variation of *E. granulosus* carriage in wild dog populations. Quantitative burdens of *E. granulosus* suggest parasite aggregation is present with just 15.8% of peri-urban wild dogs sampled responsible for 66.7% of the total worm burden. On average, bitches were found to have much higher IPC than male dogs, and the average IPC generally decreased with age, particularly >2 years. Significant geographical variation was found in the prevalence of *E. granulosus*, with a strong propensity for clustering. The average size of clusters was 22.5 kms. No physical environment or climate variables examined were significantly associated with the prevalence of *E. granulosus*. The fitted model accounted for the majority of the spatial dependence in prevalence after fitting individual and climatic variables. This is the first study to present a prediction map of *E. granulosus* prevalence in peri-urban wild dogs in Australia. Our predictive prevalence map indicates that *E. granulosus* is highly endemic in the southern region of the Gold Coast local government area (LGA), most of the Byron Bay region and surrounding LGA's, and the south-west corner of the Sunshine Coast LGA. The prediction map focuses on the most significant sex/age category of peri-urban wild dogs of prevalence of *E. granulosus* for potential environmental / public health impact and provides a useful tool for targeted disease management strategies in peri-urban areas. Targeting risk mitigation strategies is important as broad scale management of wild dog populations is difficult to implement.

INTRODUCTION

Echinococcus granulosus is a common zoonotic pathogen worldwide (Jenkins et al., 2005, Deplazes et al., 2017). In intermediate hosts (e.g. macropods, livestock), it presents as hydatid cysts in the internal organs, with humans acting as accidental hosts (Jenkins, 2006). In Australia, transmission occurs through a predator-prey relationship, with dog-sheep, and/or dog-macropods comprising the primary domestic and sylvatic cycles, respectively. Wild canines consume the cysts of infected intermediate hosts, and as definitive hosts of the tapeworm, shed eggs into the environment which become infective to intermediate hosts, and the cycle continues (Jenkins and MacPherson, 2003). *E. granulosus* remains common in canine populations around Australia, particularly in wild dogs (Baldock et al., 1985; Grainger and Jenkins, 1996; Jenkins et al., 2008; Jenkins and Morris, 2003) but also rural farm dogs (*C.l. familiaris*) (Jenkins et al., 2014a) and occasionally in foxes (*Vulpes vulpes*) (Jenkins and Craig, 1992). Wild dogs are known to carry large worm burdens in comparison to foxes, and as a result are seen to be the most significant definitive host in terms of transmission in Australia (Mackenstedt et al., 2015).

Human infection results in the development of fluid filled cysts that gradually impact on the internal organs, most commonly the liver and/or lungs. In the 1950's and 60's certain areas of Australia had some of the highest rates of human hydatid disease in the world (Beard, 1964). Those residing in rural areas were at much greater risk of becoming infected, with 7.8 cases per 100,000 population compared to the Australian annual rate of 1.6 cases per 100,000 population (O'Hern and Cooley, 2013). A hydatid control program was implemented in Tasmania, in 1962. The program focused around the prohibition of feeding offal to farm dogs, education regarding hygiene, as well as the monitoring and treatment of dogs. This resulted in a fast and significant reduction in the presence of the pathogen in dogs, sheep and humans, significantly aided by the absence of sylvatic cycles. As a result Tasmania was declared hydatid free in 1996. Unlike Tasmania, the presence of feral dogs and dingo-hybrids proved a significant hurdle towards similar success for the control of hydatid disease on the mainland. Despite the successful implementation of a control program and increased awareness of the disease, new cases of hydatid disease are still diagnosed in Australia every year. Serological testing at Sullivan Nicollaides (pathology laboratory) has seen on average 7.9 positive patients per year between 2005 - 2017 (Jenny Robson, Sullivan Nicollaides, Brisbane, unpublished data). Many cases are likely to have been acquired overseas although cases from Australia do occur (David Looke, Infection Management Services, Princess

Alexandra Hospital, Woolloongabba, personal communication). A rare case of hydatid infection in an urban child, whose only risk factor was an occasional bicycle ride within rural areas where wild dogs are present, was recently recorded (Clare Nourse, Children's Health Queensland, Lady Cilento Hospital, South Brisbane, personal communication). However, it remains likely that the current presence of the disease within the Australian public is under-acknowledged. Only 40% of cases were notified in Tasmania between 1996 - 2012 (O'Hern and Cooley, 2013) and cases have also been considered underreported in New South Wales and the Australian Capital Territory where just 17 of 321 cases were notified (Jenkins and Power, 1996).

Incursions of wild dogs into peri-urban and urban environments have emphasised the importance on the pathogen. With novel data showcasing movement and home-range patterns of peri-urban wild dogs, our understanding of potential impacts to human health is developing (Allen et al., 2013; McNeill et al., 2016). Within peri-urban areas, *E. granulosus* is maintained through a predator-prey relationship between wild dogs and susceptible intermediate hosts such as macropods. Swamp wallabies have been shown to be a staple dietary item of peri-urban wild dogs (Allen et al., 2016, see chapter 4) and approximately half of peri-urban wild dogs across SEQ and northern NSW carry the tapeworm within their intestines (see Chapter 3). However such high prevalence does not occur in all wild dog populations, with far lower detection of the pathogen in far north Queensland (Brown and Copeman, 2003). It is likely there are numerous other potential influencing factors that contribute to maintenance of the parasite. In particular, climate and physical environment contributors, as well as potentially the individual level factors of each dog such as age and sex. Knowledge of these risk factors not only allows the opportunity to mitigate such risks within management programs but also presents an opportunity to develop targeted interventions to ensure the most efficient allocation of control strategies.

This study investigates the geographical distribution and risk factors of *E. granulosus* within the peri-urban wild dog population across south-east Queensland and northern New South Wales. Using model-based geostatistics and data on individual dog factors, physical environment and climate, we investigate the spatial variation in the prevalence of the parasite within the peri-urban wild dog population. With the primary aim of quantifying the geographical variation in *E. granulosus* prevalence and its determinants in this study, we also combined prevalence data with data on the intensity of infection and estimate the Index of

Potential Contamination to profile age and sex groups and their ability to contribute to disease transmission. Together, this information will provide evidence for the design of management strategies targeting areas most at risk.

MATERIALS AND METHODS

Trapping locations

The study area has previously been described in Chapter 3. Two hundred and one (201) wild dog carcasses were supplied through council or private pest management programs undertaken within the study area between August 2012 and May 2015. All wild dogs were culled as part of routine pest management programs, and the supply of carcasses approved for necropsy by the University of Queensland Animal ethics committee (approval number SVS/145/13).

Sources of data

Pathogen data

The data collected on the presence of pathogens from the cross sectional survey conducted in Chapter 3 were utilised. To briefly summarise: Intestinal contents were scraped into a dish and sieved. Adult *E. granulosus* worms were expelled into a 1000 mL beaker and two 50 ml subsamples were counted under a dissecting microscope, with the subsequent counts corrected to provide the number of worms per 1L of intestinal content (Jenkins et al., 2008). The Index of Potential Contamination (IPC) was calculated using the methods presented in schistosomiasis studies (Vercruysse et al., 2001) and adapted for use with worm burden estimates. IPC considers both prevalence and intensity. Where previous studies have applied egg counts (eggs per gram of faeces) to calculate IPC, we have applied worm burden data. Adaption of IPC to *E. granulosus* data allows for comparison of age and sex groups on their potential to contaminate the environment through their ability to shed infective eggs according to their worm burden. Animals with higher worm burdens likely have the ability to shed a greater number of infective eggs into the environment so, it is important to consider the intensity of infection as well as prevalence.

Wild dog ages

At necropsy, the top jaw was removed and stored at 4 °C until processing. Jaws were boiled for approximately two hours. Once cool, the canine teeth were extracted. Teeth were examined via x-ray (exposure settings 48 KVP 1.25 mAs) at the University of Queensland Veterinary Medical Centre. Individual teeth on the x-ray film were analysed digitally using

the ‘measure line segment’ tool in SYNAPSE PD-S viewer (Fujifilm®) according to the methods of Knowlton and Whittemore (2001). Dogs were then back aged to provide an estimation of age in months as described in Kershaw et al. (2005).

Climate data

Rainfall data was provided from the Bureau of Meteorology (www.bom.gov.au). Relative humidity (%) and Temperature (°C) were provided by collaborating partners Biosecurity Queensland. Data was provided for the states of Queensland and New South Wales.

Allocation of home-ranges to point data and data extraction from GIS

GPS tracking information of 28 peri-urban wild dogs from within the study area were provided by Biosecurity Queensland and this data has recently been published (McNeill et al., 2016). Home-ranges were calculated using minimum convex polygons in preference to kernel densities to ensure that the complete range of contact with different environmental sources were accounted for within the analyses. Home ranges were then allocated to individual waypoints of wild dog captures according to similarities of age, sex and season. The centroid of a home-range was placed on top of the point data and allocated a matching ID. This was conducted for all peri-urban wild dogs (n=201). Vector layers were first converted to rasters prior to extracting the values for both points and home-ranges. For each raster layer, data was extracted at each point utilising the ‘extract values to table’ spatial analyst tool in ArcGIS. The average of each home-range was also extracted utilising the ‘zonal statistics as table’ tool. The extracted values for point and polygons were correlated using statistical software (STATA). A Pearson’s correlation coefficient greater than 0.7 was accepted as equivalent and the point data was selected to continue further analyses. All data was standardised by taking each value, subtracting the mean and dividing by the standard deviation for each category. The standardised values were then used in the analyses. Extractions of the independent variables were conducted in ArcGIS (ESRI, ArcGIS Desktop 10.3).

Non-spatial statistical analysis

We investigated the spatial epidemiology of *E. granulosus* prevalence using the spatial analysis pipeline previously described by Magalhaes et al. (2011). To investigate the role of physical environment and climate in the prevalence of *E. granulosus* carriage in wild dogs, non-spatial univariable and multivariable logistic regression models were utilised. Nine

independent variables were selected, including climatic factors (maximum rain, minimum rain, average rain, minimum relative humidity, maximum relative humidity, minimum temperature, maximum temperature), and significant physical landscape factors (distance to roads, distance to natural waterways). Independent variables for the climatic factors were calculated according to wet (November to March) and dry (April to October) season. Univariable non-spatial generalised linear model (GLM), (family: Bernoulli, link: logit) were developed using *E. granulosus* presence as the response variable, age and sex as individual factors, with the physical environment variables and climate data as predictors. Correlations between climatic covariates were investigated using Pearson's correlation coefficients. Variables significant at $p < 0.20$ were further considered in a multivariable model. The final multivariable model was determined by a backwards stepwise regression. Statistical analyses for the univariable and multivariable models were conducted in STATA/IC 13.1 (*Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP).

Analysis of spatial dependence

The extent of geographical clustering was quantified using a semivariogram. Semivariograms are utilised to describe the extent of spatial dependence in point data. A semivariogram is described by three parameters: the nugget, the partial sill and the range. The nugget represents the variance in the data that is due to non-spatial attributes (e.g. measurement error or random variation). The partial sill represents the variance in the data that is due to factors that determine spatial clustering. The range is the distance at which spatial correlation ends and signifies the average size of clusters of the pathogen. An empirical semivariogram was developed utilising the raw prevalence data to determine the extent of geographical clustering and the proportion of variance due to spatial factors. A maximum distance of approximately 55 km (0.5 decimal degrees) was utilised representing half the distance of the short side of the area. Residuals from the final multivariable model were produced and used as input to develop a residual semivariogram to address the implications on fitting individual factors, physical environment and climate on the geographical clustering of *E. granulosus* prevalence. Semivariograms were developed utilising the geoR package of R software (The R Foundation for Statistical Computing, Version 3.3.3).

Predictive mapping of E. granulosus prevalence

Based on the results of the residual semivariogram (Figure 5.2) geostatistical models were built to account for residual spatial variation. A model-based geostatistical Bernoulli model

of *E. granulosus* prevalence was built in OpenBUGS. This model included all variables included in the final multivariable non-spatial logistic regression model (maximum rain, average rain, maximum relative humidity, and maximum temperature) individual-level covariates (age and sex) and a geostatistical random effect that accounts for spatial autocorrelation between pairs of locations of wild dogs (measured as by longitude and latitude). The parameter Phi indicates the rate of decay of spatial correlation (measured in decimal degrees, and $3/\text{Phi}$ determines the cluster size; where 1 decimal degree is approximately 111 km at the Equator). The parameter Tau indicates the variance of spatial random effect. Prediction locations and data values were then mapped and extracted in the GIS to be included in the final prediction model. The outputs of the Bayesian model are known as ‘posterior distributions’ and include the parameter estimates and spatial prediction at non-sampled locations. The posterior distributions represent uncertainties that are associated with the parameter estimates. The mean of the posterior distribution and the standard deviation of the prediction were mapped in the GIS. Female peri-urban wild dogs within the 0-6 month age category were chosen as the age and sex category for predictions, due to their significantly higher worm burdens.

RESULTS

Peri-urban wild dog characteristics

Males and females were equally represented within the sampled population. Dogs aged below 12 months old were most frequent within the population (57%) with 1-2 year olds, 2-5 year olds and greater than 5 year olds represented at 22%, 10% and 10% respectively. Wild dogs were captured throughout all months of the year with 58% captured during the wet season and 42% captured during the dry season. Coat characteristics of wild dogs ranged from ‘typical’ ginger colour to; black, black and tan, black and white, white and tan, as well as mixed brindle. Variation in tail lengths and ‘bushiness’ were also featured.

Echinococcus granulosus prevalence and intensity of infection

Wild dogs infected with *E. granulosus* were caught throughout all regions of south-east Queensland as well as the Byron Bay Regional and Lismore City council zones in north-eastern New South Wales. Geographical locations of infected and non-infected wild dogs are shown in Figure 5.1. Small intestines from 201 wild dogs within the study area were examined. In total, 101 (50.7%) individuals were infected with adult worms within their intestines. An undetermined proportion of tapeworms had gravid segments. Worm burdens

recovered from the wild dogs ranged from 40 to 85,950 worms (Table 5.1). The majority of dogs sampled (~73%) carried less than 10,000 worms each, which represented only 16.9% of the total worms recovered. A much smaller proportion (15.8%) of dogs carried above 20,000 worms, which represented nearly 70% of total worms recovered. The total adult worm burden carried by infected wild dogs was 998,856.

Table 5.1 Distribution of pathogen intensity in peri-urban wild dogs across south-east Queensland and north-eastern New South Wales

Group	Worm burden	Group size (% of infected animals)	No. of worms carried by each group (% worms recovered)
1	1-999	30 (29.7%)	9920 (1%)
2	1000-9999	44 (43.6%)	158905 (15.9%)
3	10000-19999	11 (10.9%)	164180 (16.4%)
4	20000-90000	16 (15.8%)	665860 (66.7%)

Table 5.2 Index of Potential Contamination for *E. granulosus* for male and female peri-urban wild dogs

Sex	n (dogs)	n (infected)	Prevalence (%)	Worm Burden (GM+)	Crude IPC	Relative IPC (%)
Male	97	51	52.6	1785.3	93865	34.23
Female	101	48	47.5	3794.4	180328	65.77

Table 5.3 Index of Potential Contamination for *E. granulosus* across 5 age categories

Age Group	n (dogs)	n (infected)	Prevalence (%)	Worm burden (GM+)	Crude IPC	Relative IPC (%)
<6 months	42	22	52.4	4299.2	225195	33.9
6-12 months	56	25	44.6	3041.6	135784	20.5
1-2 years	36	18	50.0	3552.0	177602	26.8
2-5 years	21	12	57.1	1085.0	61999	9.3
>5 years	19	12	63.2	995.2	62854	9.5

Male and female wild dogs were equally likely to be infected with the *E. granulosus*. However, females carried much higher worm burdens, and the Index of Potential Contamination (IPC) suggested that females contribute 66% of the potential contamination by *E. granulosus* (Table 5.2). Age was not a risk factor in the potential for being infected with the tapeworm. However, IPC values indicate that wild dogs greater than two years of

age have a much reduced contribution to environmental contamination compared to younger animals (Table 5.3).

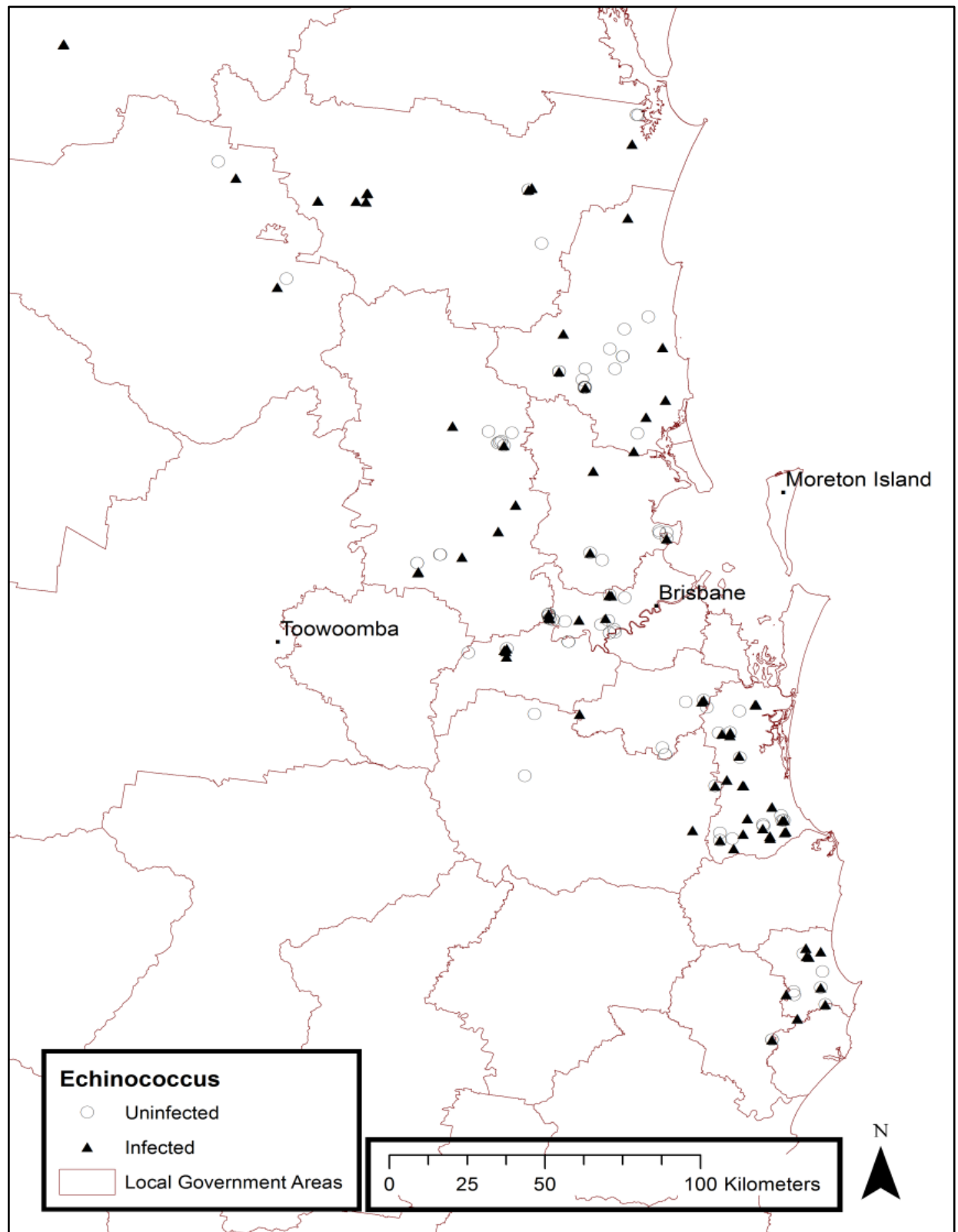


Figure 5.1 the geographical locations of trapped peri-urban wild dogs with (▲) or without (○) *E. granulosus* infections.

Spatial dependence in *E. granulosus* prevalence

The raw semivariogram shows a significant tendency for *E. granulosus* clustering, with 14% of spatial variance explained by geographical location (Figure 5.2A). The semivariogram of the model residuals shows less spatial clustering after fitting the climatic variables (Figure 5.2B). This explains most (92%) of the spatial variation, suggesting that other factors remain unaccounted for in the model.

Bayesian model-based geostatistical model of *E. granulosus* prevalence

Table 5.4 presents the estimates of posterior mean for *E. granulosus* prevalence. After accounting for the covariates, the radii of the clusters were 22.5 km. Our results indicate that males were at higher risk of infection compared to females, and wild dogs aged between 1-2 years old were at higher risk of infection compared to wild dogs aged 0-6 months. However, there were no significant associations between physical environment or climate variables (maximum rain, average rain, maximum relative humidity or maximum temperature) and the prevalence of *E. granulosus* infection in peri-urban wild dogs.

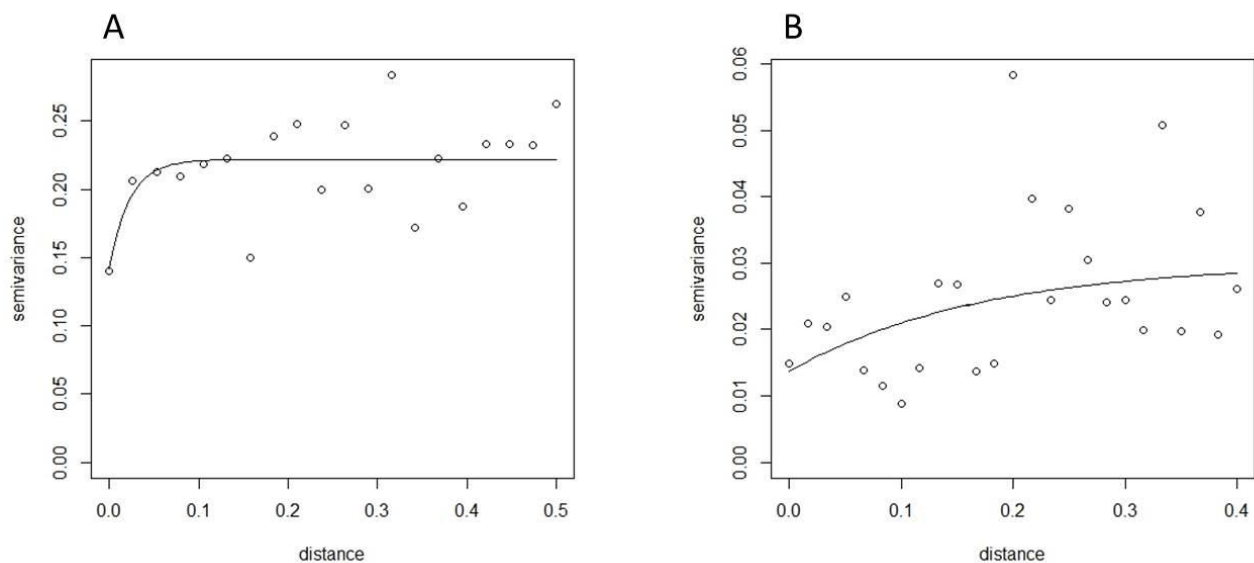


Figure 5.2 (A) Raw semivariogram (B) Residual semivariogram. Distance is measured in Decimal Degrees

Table 5.4 Estimates of posterior mean (in the log odds scale) for *E. granulosus* prevalence across south east Queensland and northern New South Wales, based on Bayesian geostatistical logistic regression models.

Variable	Posterior mean (95% CI)
Female (vs male)	-0.61 (-0.58, 0.02)
6-12 months (vs <6 months)	-1.17 (-1.05, 0.05)
1 - 2 years (vs <6 months)	0.22 (0.03, 0.19)
>2 years (vs <6 months)	-0.13 (-0.08, 0.035)
Maximum Rain	1.6 (0.17, 1.36)
Average Rain	-1.02 (-0.80, 0.15)
Maximum RH	0.59 (0.02, 0.56)
Maximum Temperature	0.39 (0.02, 0.36)
Intercept	0.89 (0.05, 0.82)
Rate of decay of spatial autocorrelation (Phi)	15.21 (0.15, 16)
Variance of spatial random effect	0.89 (0.04,1.13)

The predictive map of *E. granulosus* infection indicates that prevalence tends to increase from the west towards the eastern coast line (Figure 5.3A). Clusters of high prevalence of *E. granulosus* infection (prevalence >85%) are present in the south-west corner of the Gold Coast (Queensland) and a significant proportion of the Byron Bay Regional Council area, expanding into the Tweed Coast Region of New South Wales. Another cluster of high prevalence was predicted north of Brisbane in the south-west corner of the Sunshine Coast Hinterland Region. The areas predicted with high prevalence correlate with areas of low uncertainty represented by small standard deviations (Figure 5.3B). Large areas of moderate prevalence (>55-70%) correlate with areas of high uncertainty, represented by large standard deviation.

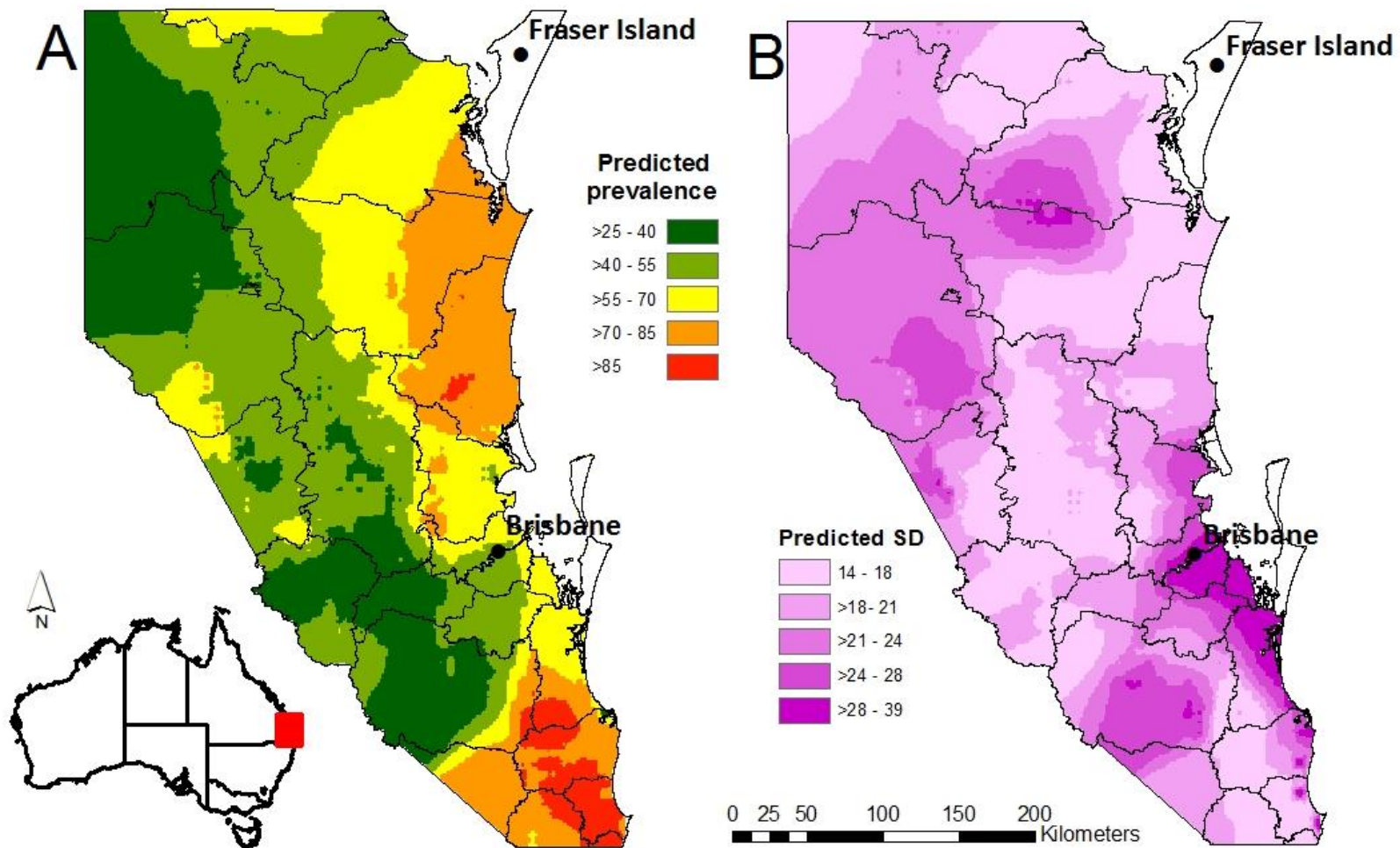


Figure 5.3 (A) Predicted mean prevalence of *E. granulosus* infection in female peri-urban wild dogs aged 0-6 months. Estimates are the mean posterior predicted prevalence values. (B) Predicted standard deviation

DISCUSSION

This study confirms that *E. granulosus* carriage by peri-urban wild dogs is common in south-east Queensland and surrounding areas. Importantly, our results demonstrate that *E. granulosus* infection risk in peri-urban wild dogs is remarkably clustered within defined areas in Queensland. Recent information surrounding wild dog spatial use in peri-urban and urban environments has concluded that management objectives should be focused towards targeted control of individuals or small groups, in comparison to a broad scale (nil-tenure) approach where an overall reduction in population is the aim (McNeill et al., 2016). The research presented here provides important information for mitigating the potential impacts on public health from wild dog carriage of *E. granulosus* where targeted management can be implemented to achieve a reduction in predicted risk.

Studies have assessed age and sex risk factors in several species of intermediate hosts including macropods (Barnes, 2007), cattle (Gemmell and Brydon, 1960; Pullar and Marshall, 1958), pigs (Gemmell and Brydon, 1960), and sheep (Gemmell et al., 1986). However, data relating to risk factors in wild dogs was not available. Generally, male canines have a higher risk of infection with parasites, and prevalence decreases with age (Coggins, 1998; Nijse et al., 2015; Payne-Johnson et al., 2000; Visco et al., 1977a). The results presented in this study suggest that males were at higher risk of infection than females although this was not statistically significant. It is likely the most common source of *E. granulosus* infection in peri-urban wild dogs is through the consumption of intermediate hosts. Due to high resource availability and smaller home ranges of wild dogs in peri-urban areas, males and females are likely to have similar exposure to prey species whereby they become infected. Despite no difference in prevalence, there were obvious differences in parasite aggregation between the sexes. While previous studies have identified parasite aggregation in wild dog populations, sex was reported as being unimportant (Jenkins et al., 2008). In contrast, our data suggests that males are driving prevalence, while females are driving IPC, due to their high infection intensities. Our data also suggests that younger wild dogs have larger worm burdens and have greater potential to contribute to environmental contamination than older animals. Such high worm burdens in bitches and pups may be related to the feeding behaviour of wild dogs. Dominant females often have first access to the carcass and as a result will consume the preferred organs, such as liver and lungs of the prey (Lee Allen, Biosecurity Queensland, personal communication). Males consume the remaining tissue which is less likely to be infected by the intermediate stage of the parasite and hence, have less exposure for infection. Females will also regurgitate their food to feed their young pups (Thomson, 1992) which may explain the high worm burdens found in younger animals. Not only is the feeding behaviour of wild dogs important but also, their selection of prey.

Wallabies, particularly swamp wallaby (*Wallabia bicolor*), have been shown to be a commonly consumed prey item (Allen et al., 2016; Robertshaw and Harden, 1985) and consumption has been shown to be positively associated with *E. granulosus* infection in peri-urban wild dogs (see Chapter 4). Hence, both dietary preference and feeding behaviour are likely to play a large role in the prevalence of pathogens and presence of parasite aggregation within peri-urban wild dog populations. There is also data to propose evidence of age-related patterns (Budke et al., 2005; Lahmar et al., 2001; Moro et al., 2005), suggesting resistance to re-infection could be present in dogs (Torgerson, 2006). It was unknown if the development of resistance to *E. granulosus* in dogs is based on natural genetic mechanisms or because of an immune response (Herd, 1977). More recent research has shown that parasite specific IgE and local IgA may be related to the protection of dogs against *E. granulosus* (Moreno et al., 2004). However, it is clear that more research is required to understand the mechanisms of resistance to re-infection dogs (Torgerson, 2009). The application of the index of potential contamination (IPC) to *E. granulosus* assists in understanding the role of age and gender on environmental contamination. Additionally, it also provides a method for comparison of parasite burden across different study areas and it allows for simplified mapping of intensity. The data from this study utilised many individual locations for sampling, resulting in high proportion of the data set presenting with an IPC of 0. However, future studies could supplement this.

Climatic variables have long been known to influence the prevalence of infectious pathogens in animals (Gordon, 1948). However, the thick walled characteristics of *E. granulosus* eggs means that they are highly resistant to adverse climatic effects (Thevenet et al., 2005) and desiccation and reduced viability is less likely to occur in comparison to thinned shelled eggs or oocysts of other parasites (King et al., 2005; Zhang et al., 2012). Climatic variables have been reported to influence helminth prevalence in red foxes in Western Australia (Dybing et al., 2013). However, the study conducted by Dybing et al. (2013) did not detect *E. granulosus* in their sampled fox population and their results did account for spatial variation. Previous studies have shown that climate and geographical conditions could not explain variation in the availability of *E. granulosus* eggs in the environment (Chaabane-Banaoues et al., 2015). It is possible that the climatic variables within our study area and prediction zone did not vary enough to detect climatic risk factors. The sub-tropical climatic conditions present in south east Queensland and the characteristics of *E. granulosus* eggs may allow for easy survival in the environment. The observed prevalence of *E. granulosus* infection was highly spatially auto correlated which was significantly reduced once the climatic co-variables were fitted. However, not all variables were accounted for and some minor spatial dependence remained. Spatial dependence is a common phenomenon in ecology where sampled values located

nearby are more similar than those further apart (Dormann, 2007). Spatial dependence may be reliant on exogenous or endogenous factors (Dormann, 2007). Exogenous factors include climate and other physical environmental structures. Endogenous factors are those often influenced by the biology or ecology of the host species, and are typically more difficult to quantify. Although consideration was given to the variation in home-ranges of peri-urban wild dogs, there are no data available on wild dog densities suitable for model input. The lack of standard measures and methodological inconsistencies for wild dog populations makes it difficult to produce density estimates (Allen et al., 2011). Further attempts to reduce spatial dependence in the current dataset are hampered given the lack of data on density and other unpredictable factors, such as individual behaviour of wild dogs and influence on use of landscape features for denning sites, that may influence results.

This is the first study to present a prediction map of *E. granulosus* prevalence in peri-urban wild dogs in Australia. The prediction maps show clusters of increased / high predicted prevalence in the southernmost part of the study area, within the Gold Coast, Tweed Shire and Byron Bay local government areas. Another cluster with predicted likelihood of high prevalence is the south-west corner of the Sunshine Coast Council. The locations with high human density are mostly represented by the areas with moderate prevalence but also high uncertainty of prediction. These areas require further investigation as they present the highest potential for public health impact, given the higher likelihood of wild dog:human interactions. The western areas of the prediction map, where human density decreases, presents the least risk of infection. These zones start expanding into a more rural landscape, where wild dog home-ranges may also increase. Hence, the prediction map is based on mean spatial predictions rather than the sampled data. However, wild dog densities are probably also reduced in such areas. The distribution of risk is unlikely to differ between ages and sex, but, the endemicity will. The risk map presented in this study should be considered be a conservative estimate of the predicted prevalence of *E. granulosus* within the population of peri-urban wild dogs, and focuses on the most significant sex/age combination for potential environmental / public health impact. This is a more useful tool for targeted disease management in peri-urban environments where broad scale management of wild dog populations are difficult to implement.

This prediction map can be utilised to mitigate impacts on targeted groups of peri-urban wild dogs in contrast to population level reductions across the landscape. Management programs for rural wild dogs utilise a nil-tenure approach where the strategy for control is based on reduction in overall population numbers (WoolProducers, 2014). Wild dogs living within peri-urban regions are capable

of residing within small fragments of urban bushland and are known to have flexible spatial requirements (McNeill et al., 2016). Traditional methods of control are often limited within peri-urban zones, due to legislative restrictions on the use of toxins (APVMA, 2008). Importantly, reductions in overall population levels may not have a significant impact on the specific individuals or small groups of wild dogs that post the greatest risk to public health. To manage specific impacts of potential public health issues from *E. granulosus*, targeted control should be implemented in the predicted high risk zones. However, culling of wild dogs may not necessarily be the most effective method to cause a reduction of infective eggs in the environment. Maintenance of fox populations with targeted praziquantel baiting has proven to be effective in significantly reducing the presence of *E. multilocularis* eggs within high risk urban environments across Europe (Hegglin et al., 2003). The predicted areas of high prevalence in south east Queensland would provide an ideal landscape for targeted praziquantel baiting to reduce *E. granulosus* prevalence within the peri-urban wild dog population.

Previous research into pathogens carried by wild dogs has considered the presence of *E. granulosus* within northern Queensland (Townsville) urban wild dog communities to be negligible (Brown and Copeman, 2003). The unfavourable climate (in Townsville) as well as the detection of low worm burdens, is likely to explain their conclusions. By contrast, wild dogs captured within the Maroochy shire (now a part of the Sunshine Coast Region) were found to frequently carry high worm burdens (Jenkins et al., 2008). Combined with residing in conditions favourable to *E. granulosus* egg-survival, the risk posed towards public health impacts is much more substantial (Mackenstedt et al., 2015). However in contrast to our findings, infected wild dogs detected by Jenkins et al. (2008) were found to reside in the most rural regions of their study area. In contrast, our findings suggest that potentially the turnover of wild dogs within peri-urban regions is not as frequent as previously suggested as we report an increase in prevalence of *E. granulosus* in peri-urban zones. Data has shown that wild dogs have the ability to persist in high-risk urban environments (McNeill et al., 2016). Limited control may facilitate social groups to have stabilised populations within these zones, enabling sustained predation on common intermediate hosts such as macropods. Alternatively, the pathogen once thought to be isolated to rural regions has slowly dispersed and is now more widespread than previously detected, potentially driven by human environmental modification (Bradley and Altizer, 2007; Daszak et al., 2001). However, understanding the potential risk to humans is complicated. Direct contact between humans and wildlife is often limited, especially within urban regions. Contact between wild dogs and domestic dogs have not been quantified in urban areas. In rural areas of New South Wales variation in temporal activity levels caused no known interactions between domestic and wild dogs, however, there was evidence to

suggest there was spatial overlap (Sparkes et al., 2016b). Hence, in peri-urban areas it is highly likely that wild dogs and domestic dogs may overlap spatially, and reports from the community indicate direct interactions between wild and domestic dogs are not uncommon (Gentle et al., in preparation). Data on peri-urban wild dog movements and home-ranges within south-east Queensland (Allen et al., 2013; McNeill et al., 2016) has revealed the use of town parklands, school grounds, residential yards as well as adjacent areas of bushland and other corridors of vegetation. These recent findings are potentially significant in terms of hydatid disease transmission to humans and in particular children. An emerging epidemic of human alveolar echinococcosis was detected in Switzerland 10-15 years after an increase in urban fox population (Schweiger et al., 2007). Although current evidence of hydatid disease in humans suggests that transmission is rare, it can take several years to manifest and detect. Hence, long term surveillance and reporting of new cases in urban patients is required to understand if there are any developing impacts from such recent high prevalence within the peri-urban wild dog population.

This study's findings are important but need to be interpreted within the context of some limitations. Firstly, despite our best efforts, we were unable to collect samples from across all landscapes, given the reliance on captures from current management programs which are often conducted in response to public complaints. Secondly, the prevalence risk map is a conservative estimate and does not present the worst case scenario; rather, it represents the sex age combination of the most significant risk group based on IPC. Thirdly, a map of IPC could not be produced due to the dispersal of the data set. Mapping the IPC would identify the highest potential environmental contamination areas and hence, would provide an additional, valuable tool for implementation of targeted management programs. Future research should consider their ability to implement mapping of IPC in their initial study design.

In conclusion, our results demonstrate that *E. granulosus* is common in peri-urban wild dog populations across south east Queensland and surrounds. Despite males driving prevalence, it seems that females are the main drivers of IPC and hence have greater potential to contribute to environmental contamination. Prevalence in wild dog populations is not associated with the physical environment or climate however, they do account for the majority of spatial dependence in the data. The data presented here provides information on the locations of clusters with high predicted prevalence. These locations can be targeted with management programs in an attempt to reduce the high prevalence in the wild dog population and reduce the risk of transmission to humans or domestic dogs. Continual monitoring of prevalence and intensity in wild dogs is important to understand the status of the parasite in the definitive host. It would also be beneficial for future

research to understand the greater lifecycle of the parasite, through environmental sampling of soils within the areas of high and low predicted prevalence.

CHAPTER 6

Geographical distribution and risk factors for
hookworm carriage in peri-urban wild dog
populations

ABSTRACT

In Chapter 3, hookworms were identified as a common pathogen carried by peri-urban wild dogs. In Chapter 4, the association of diet composition with pathogen presence was investigated. Following this, further investigation into the role of physical environment, climate, and individual dog level factors in the geographical variation of the probability of hookworm infection by wild dogs was conducted. While none of the physical environment factors were statistically significantly associated with hookworm presence, maximum rainfall was negatively associated with hookworm carriage. Significant spatial variation in the probability of hookworm carriage was not identified, suggesting the propensity for clustering is very weak. A predictive map of the probability of hookworm carriage was generated in a geographical information system. The predictive map indicates that the probability of hookworm carriage in wild dogs is highly likely in the south western regions of the study area, as well as on the southern side of the Brisbane River in the Brisbane City Council local government area. Overlap between the predicted probability of hookworm and *Echinococcus granulosus* carriage suggests that in areas with predicted high probability of hookworm carriage, management programs to control both parasites simultaneously could be achieved with more efficiency. The findings of this chapter compliment the previous three research chapters in that the predictive hookworm map provides a useful tool for geographically targeted and integrated disease management options in peri-urban regions when utilised alongside the prediction map of *E. granulosus* presented in Chapter 5. Together, the data from Chapter 5 and Chapter 6 can assist with mitigating the impacts of zoonotic parasitic disease in peri-urban environments where a targeted approach to control can be applied. This approach will assist in allocating limited management resources to the most affected areas.

INTRODUCTION

Hookworm infections in domestic dogs and wild canines are common around the globe (Seguel and Gottdenker). Across Australia, the prevalence within the domestic dog population is estimated to be 6.7% (Palmer et al., 2007), which is likely to reflect regular anthelmintic treatment provided by owners to their pets. However, wild dog populations are not part of deworming programs and current information regarding the abundance and distribution of hookworms within these populations is limited.

All four canine hookworm species, *Ancylostoma caninum*, *Ancylostoma braziliense*, *Ancylostoma ceylanicum* and *Uncinaria stenocephala* are present in Australia (Palmer et al., 2007). Over seventy percent of wild dog scats in the northern Queensland precinct of Townsville and 100% of necropsied wild dogs were found to be hookworm-positive (Smout et al., 2013). In total, three *Ancylostoma* species were detected and although the distribution of hookworm species varied significantly dependent on location, *A. caninum* was most commonly detected overall. Similarly, within the Townsville region, Brown and Copeman (2003) also detected a high prevalence of hookworm carriage (74% of n = 27 wild dogs sampled). In sub-tropical SEQ, 37% of wild dogs were found infected with hookworms (Jenkins et al. 2008). In this study (Chapter 3), 28.8% of peri-urban wild dogs across south-east Queensland were found positive for hookworms. This prevalence is much lower than reported in northern Queensland and by Jenkins et al. (2008) in SEQ. Climatic conditions such as increased humidity and temperature are favourable conditions for hookworms and may potentially explain the variability in between the northern and southern regions of Queensland. Moreover, the lower prevalence that was reported in Chapter 3 is likely an outcome of the ability of *A. caninum* to undergo hypobiosis during the colder months (Gibbs, 1982).

The impact on human health from canine hookworm infection can vary and is dependent on the species of hookworm. All species are capable of causing some degree of skin irritation as a result of dermal migration of larvae (cutaneous larva migrans) (Hochedez and Caumes, 2007). *A. braziliense* is the only species known to produce typical manifestations of ‘creeping eruptions’. Previous research has implicated non-patent *A. caninum* as a leading cause of human eosinophilic enteritis (EE) in Queensland (Croese et al., 1994; Croese, 1988; Prociv and Croese, 1996). Infections may be asymptomatic or characterised by obscure abdominal pain, diarrhoea and peripheral eosinophilia. Many of these reports are now over 20 years old and recent information is not available. *A. ceylanicum* is now regarded as the second most common human hookworm species in the Asia Pacific Region (Bradbury et al., 2017; Inpankaew et al., 2014a). In natural, patent infections, this hookworm species has been implicated as a cause of epigastric pain, melena, diarrhoea anaemia and

peripheral eosinophilia (Brunet et al., 2015). In Western Australia, the first two cases of *A. ceylanicum* in humans presenting with ‘gastrointestinal disorders’ were diagnosed. Both patients were in their mid-twenties and had no documented evidence of overseas travel (Koehler et al., 2013). New data is required to quantify and describe the geographical distribution of hookworms within wild dog populations, particularly within peri-urban communities, so any potential impacts to public health can be addressed. Understanding the extent of geographical overlap between *E. granulosus*, also found to be highly prevalent (Chapter 5), and hookworms, could assist to inform the need for an integrated deworming protocol.

This chapter investigates the geographical distribution and risk factors for hookworm infection within the peri-urban wild dog population across south-east Queensland and northern New South Wales. This investigation aimed to model the geographical distribution of the probability of hookworm carriage using individual level and environmental factors to quantify spatial variation in the probability of hookworm carriage within the peri-urban wild dog population. This study also aimed to quantify and describe the level of geographical overlap between the predictive distribution of hookworm carriage in wild-dogs and the predictive geographical distribution of *Echinococcus granulosus*. This information can be used to inform integrative management and control programs to target the most significant areas of concern, thereby improving disease management within peri-urban wild dog populations and providing baseline data allowing for continued monitoring of these pathogens.

MATERIALS AND METHODS

Trapping locations

The study area has previously been described in Chapter 3. Two hundred and one (201) wild dog carcasses were supplied through council or private pest management programs undertaken within the study area between August 2012 and May 2015. All wild dogs were culled as part of routine pest management programs, and the supply of carcasses approved for necropsy by the University of Queensland Animal ethics committee (approval number SVS/145/13).

Sources of data

Pathogen data

The data collected on the presence of pathogens from the cross sectional survey conducted in Chapter 3 were utilised. To briefly summarise: Intestinal contents (n=201) were scraped into a dish and sieved. Adult hookworms were identified during the sieving process using morphological methods. Faecal samples (n=156) underwent floatation methods to identify the presence of eggs.

Faecal samples (n=82) also underwent PCR using previously published methods to amplify a section of the ITS region. Amplified products underwent direct digestion and / or DNA sequencing. Detailed methods are described in Chapter 3. The dataset used in this analysis only included data complete for geographical location, infection outcomes and covariates. As a result, this excluded 11% of the complete data set.

Wild dog ages

Wild dogs were aged as per the methodology presented in Chapter 5. To summarise: The top two canine teeth were removed and radiographed. Pulp cavity ratios were measured according to the published literature (Knowlton and Whittemore, 2001) and back aged to provide an estimation of age in months (Kershaw et al., 2005).

Climate data

Rainfall data was provided from the Bureau of Meteorology (www.bom.gov.au). Relative humidity (%) and Temperature (°C) data were provided by collaborating partners, Biosecurity Queensland. Data was provided for Queensland and New South Wales.

Allocation of home-ranges to point data

GPS tracking information of 28 peri-urban wild dogs from within the study area were provided by Biosecurity Queensland. Methods relating to the allocation of home-ranges to point data and the subsequent extraction of environmental variables can be found in the methods section of Chapter 5.

Non-spatial statistical analysis

To investigate the spatial epidemiology of individual-level hookworm infection status, the role of physical environment and climate was quantified using non-spatial univariable and multivariable logistic regression models. Nine independent variables (maximum rain, minimum rain, average rain, minimum relative humidity, maximum relative humidity, minimum temperature, maximum temperature, distance to roads, and distance to natural waterways) were selected. Independent variables for the climatic factors were calculated according to wet (November to March) and dry (April to October) season. Univariable non-spatial generalised linear models (GLM), (family: Bernoulli, link: logit) were developed using hookworm presence as the response variable, and age, sex, variables of the physical environment and climate data as predictors. Correlations between climatic covariates were investigated using Pearson's correlation coefficients. Variables significant at $p < 0.20$ were further considered in a multivariable model. The final multivariable model was determined using backwards stepwise regression. Statistical analyses for the univariable and

multivariable models were conducted in STATA/IC 13.1 (*Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP).

Analysis of spatial dependence

To determine the extent of geographical clustering in the probability of hookworm carriage data was assessed by a semivariogram. Semivariograms describe the extent of spatial dependence for point data and provide three main parameters: the nugget; partial sill; and the range. The nugget represents random variation, the partial sill represents non-random variation and the range signifies the average size of clusters of hookworm carriage. An empirical semivariogram was conducted with the raw data to determine the extent of geographical clustering. Semivariograms were developed utilising the geoR package of R software (The R Foundation for Statistical Computing, Version 3.3.3).

Predictive mapping of hookworm prevalence

The results of the raw semivariogram (see above) indicated that there was little spatial variation in the probability of hookworm carriage in the study area. Therefore, to develop the predictive map of hookworm carriage, the *logit* back transformation of the final non-spatial multivariable model in the ‘raster calculator’ tool of ArcGIS was applied to produce the predictive map. The following equation was used:

$$P = \frac{e^{(a+b_1 + b_2+(b_3x_3)+(b_4x_4))}}{1 + e^{(a+b_1 + b_2+(b_3x_3)+(b_4x_4))}}$$

where P is the predicted probability of hookworm carriage, a is the intercept of the final multivariable model, b_1 is the coefficient for females, b_2 is the coefficient for dogs aged 1-2 years, b_3 and X_3 are the coefficient and raster map of maximum rain respectively, and b_4 and X_4 are the coefficient and raster map of average rainfall respectively. Female dogs aged between 1-2 years were selected as the sex-age combination for prediction based on the results of the multivariable logistic regression model, which indicated that females had a higher likelihood of hookworm carriage compared to males, and also that dogs aged 1-2 years were more likely to carry the parasite compared to dogs younger than six months old. The predictive maps of hookworm carriage were constructed in ArcGIS 10.3 (ESRI, ArcGIS Desktop).

RESULTS

Dataset for analysis

Peri-urban wild dogs infected with hookworm were caught in the majority of regions across south-east Queensland as well as the Byron Bay Regional Council area in north-eastern New South Wales. Geographical locations of infected and non-infected wild dogs are shown in Figure 6.1. Males and females were equally represented within the sampled population. Across the age categories of peri-urban wild dogs sampled, animals aged six to 12 months were the most frequent within the population (31%), with dogs aged less than six months, one to two years, and greater than two years represented at 25%, 23% and 21%, respectively. Overall, 28.8 ± 7.1 % of peri-urban wild dogs were infected with hookworm. Amongst the population of wild dogs with hookworm infections, 36.4% were male and 63.4% were female. Although female wild dogs were 1.78 times more likely to be infected with hookworm than males, this was not significant (Table 6.1). 34% of infected wild dogs were classified as below six months of age, 27% were aged between one to two years, 20% were aged between six to 12 months and 18% were older than two years. However, as with sex, there was no significance amongst the age group classifications associated with hookworm prevalence. Two species of hookworm were detected; *A. caninum* and *U. stenocephala* (see Chapter 3).

Physical environment and climate factors associated with hookworm prevalence

Data indicate that hookworm carriage in peri-urban wild dogs is associated with locations with lower maximum rainfall and that this result is marginally significant ($P=0.074$) (Table 6.1). While hookworm infection is associated with increased average rainfall, this result is not statistically significant (Table 6.1). Maximum rainfall and average rainfall were both included in the final model as they were determined to be important confounders. i.e. their exclusion from the model yielded a change greater than 25% in the coefficients of the other covariates.

Spatial dependence in hookworm prevalence

The results of the empirical semivariogram for the raw hookworm infection data (Figure 6.2) demonstrated no evidence of spatial variation in the data. This finding indicated that to generate a predictive map of hookworm carriage, spatial autocorrelation does not need to be taken into account.

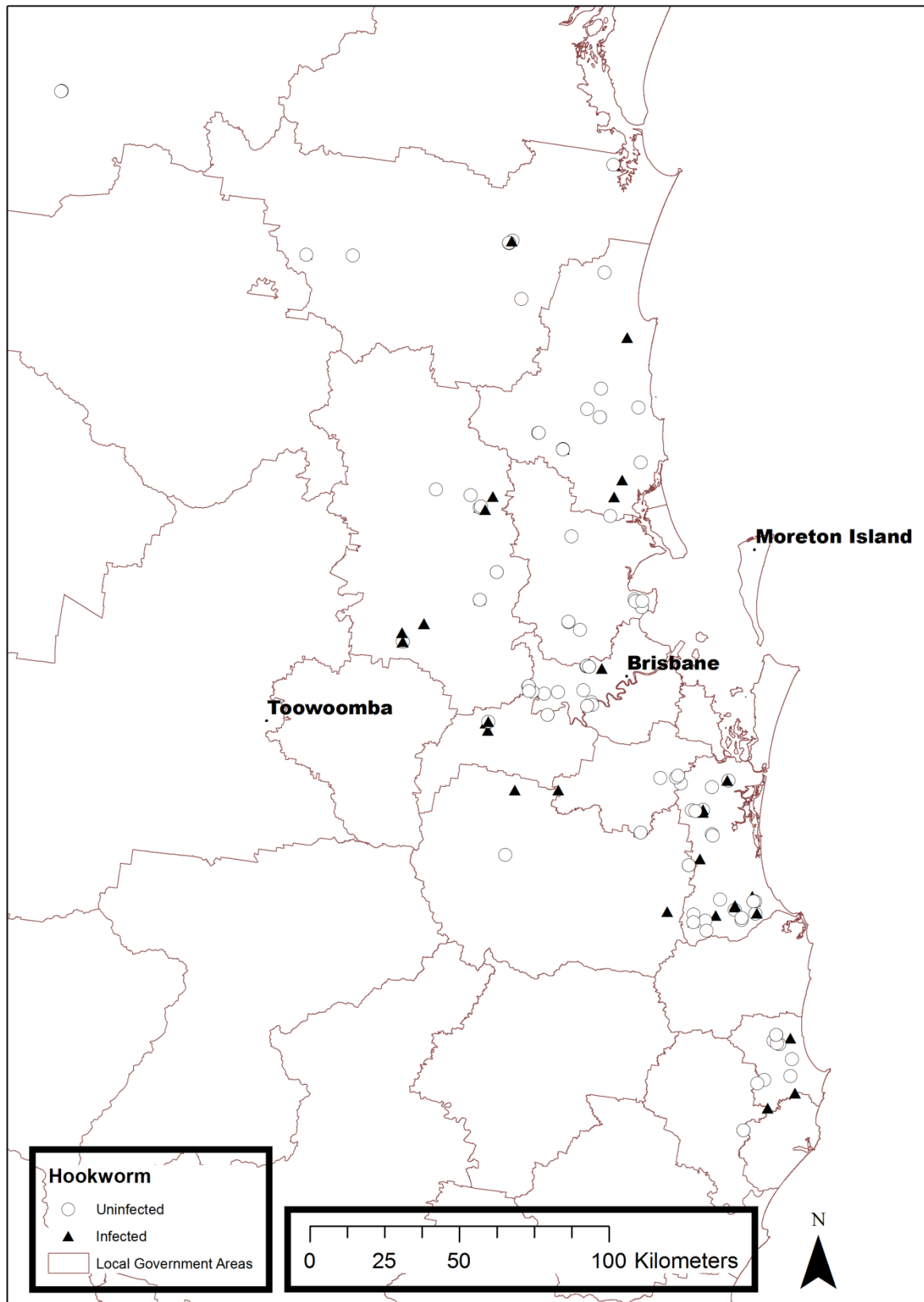


Figure 6.1 The geographical locations of trapped peri-urban wild dogs with (▲) or without (○) hookworm infections.

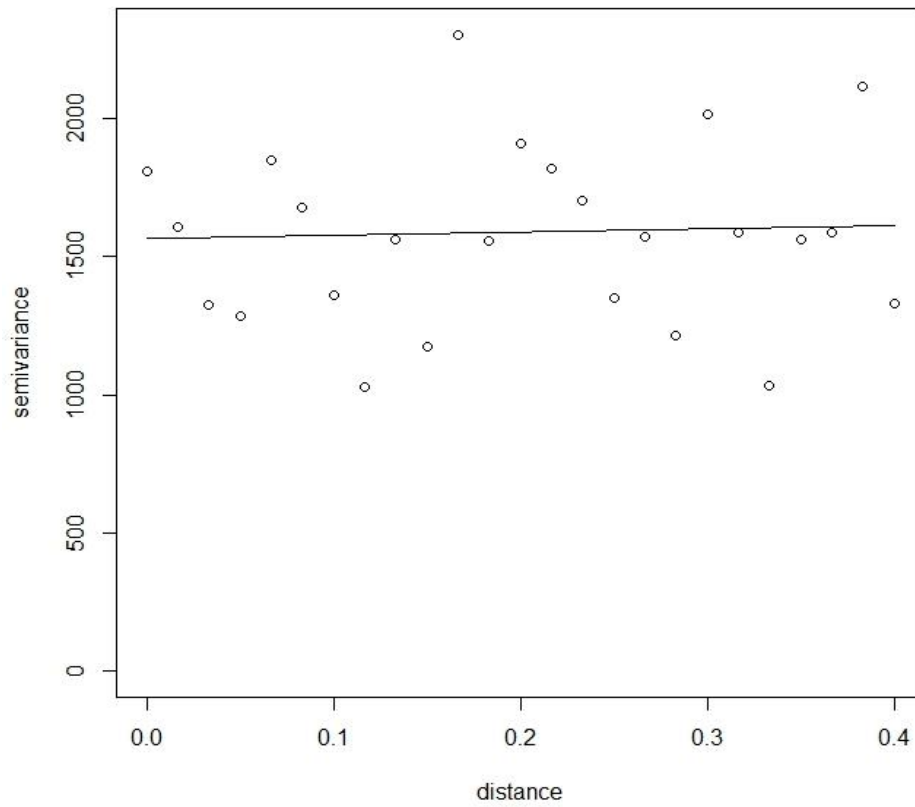


Figure 6.2 Empirical semivariogram of the raw data of hookworm infection status. Distance is measured in decimal degrees.

Table 6.1 Univariable and multivariable non-spatial logistic regression of hookworm prevalence in peri-urban wild dogs

Variables	Univariable			Multivariable		
	Odds Ratio	95% CI	P-value	Odds Ratio	95% CI	P-Value
Female (vs male)	1.83	0.87 - 3.81	0.109	1.78	0.81 - 3.95	0.153
Age 6-12 months (vs <6 months)	0.44	0.16 - 1.19	0.105	0.49	0.18 - 1.39	0.182
Age (1-2 years) (vs <6 months)	0.97	0.37 - 2.61	0.964	1.10	0.38 - 3.18	0.861
Age >2years (vs <6 months)	0.49	0.17 - 1.37	0.175	0.53	0.18 - 1.57	0.252
	Coefficient	95% CI	P-value	Coefficient	95% CI	P-Value
Dist to Natural Water	-0.20	-0.59 – 0.19	0.307			
Dist to Roads	0.11	-0.21 – 0.43	0.508			
Max Rain	-0.30	-0.67 – 0.07	0.041	-2.08	-4.38 – 0.20	0.074
Min Rain	-0.27	-0.61 – 0.07	0.125			
Avg Rain	-0.33	-0.69 – 0.03	0.072	1.75	-0.52 – 4.02	0.130
Min RH	-0.20	-0.55 – 0.15	0.254			
Max RH	-0.14	-0.51 – 0.22	0.445			
Min Temp Wet	0.06	-0.29 – 0.41	0.731			
Max Temp Wet	0.20	-0.15 – 0.56	0.262			

Predictive mapping of hookworm prevalence

The predictive map of hookworm prevalence indicated two main clusters where it is highly endemic (predicted prevalence 66 – 78%). These are located in the south west region of the study area, as well as on the southern side of the Brisbane River in the Brisbane City Council local government area (Figure 6.3A). A small cluster of high predicted prevalence (66-78%) is also evident in the north east section of the Gold Coast City Council. The areas predicted with high prevalence correlate with either low or moderate uncertainty, represented by smaller standard deviations (Figure 6.3B). Locations with predicted large standard deviations represent high uncertainty and correlate with areas of low (18-30%) predicted prevalence. Visually, comparing the overlap between the hookworm map presented in this Chapter and the *E. granulosus* prediction map in Chapter 5 suggests that regions of high predicted prevalence for *E. granulosus* correspond with regions of low predicted prevalence for hookworm. Regions of high predicted prevalence for hookworm correspond with regions that have low to moderate *E. granulosus* predicted prevalence.

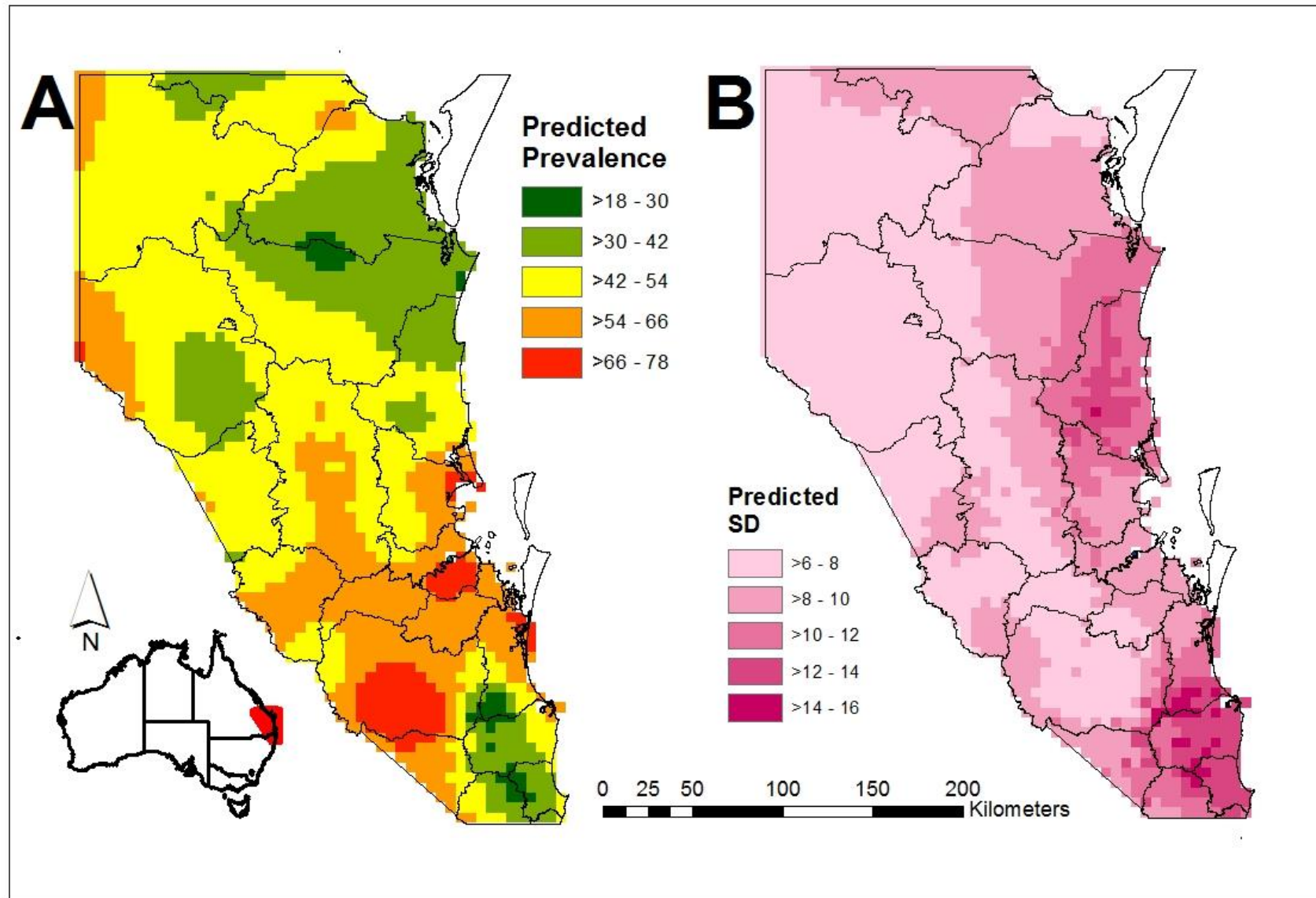


Figure 6.3 (A) Predicted mean probability of hookworm carriage in female peri-urban wild dogs aged 1-2 years. (B) Predicted standard deviation

DISCUSSION

This study provides information that allows for investigations into individual dog level risk factors that may have influenced the geographical distribution of hookworm carriage by peri-urban wild dogs. Previous studies of hookworm prevalence have either been based on small sample size collections at necropsy (Brown and Copeman, 2003; Smout et al., 2013), field collection of faecal samples (Smout et al., 2013), or only presented prevalence data (Jenkins et al., 2008). Although prevalence data can provide valuable figures for baseline information and allow for comparisons of locations, its value is limited in the development of operational programs for disease control. It is important to understand further factors that may be imperative for managing pathogens in the population.

Individual level risk factors for helminth infection in domestic dogs in Australia have been associated with animals that are less than one year old (Palmer et al., 2008). Studies in red foxes in the United Kingdom demonstrated that sex was not associated with the prevalence of hookworm in these species (Richards et al., 1995). To date, no studies have investigated the relationship between age and / or sex factors in wild dogs and hookworm infection. The results of our study indicate that while the proportion of infected females was higher than males, there was no statistical significant difference in hookworm carriage between male and female wild dogs. *A. caninum* larvae are known to rest within the tissues of hosts and then reactivate in the last weeks of pregnancy (Burke and Roberson, 1985). This contributes towards an important route of infection from mother to pup through transmammary transmission, with arrested larvae being able to infect up to three litters (Kramer et al., 2009). Our data also suggest that there was no significant difference in hookworm carriage across the age categories but that dogs aged between one and two years old were at slightly higher risk. However, we only accounted for prevalence and not quantitative egg shedding. Patent infections are known to occur in all ages of dogs (Traversa, 2012). However, due to the transmammary transmission dynamics, young pups may have the most significant impact on environmental contamination with eggs, as worm burdens and egg shedding intensities are highest in pups (Little et al., 2009; Savilla et al., 2011; Visco et al., 1977b). However, further research into understanding the age / sex association with egg shedding in peri-urban wild dogs would be beneficial because management programs could then be further targeted.

The propensity for clustering of hookworms within the study area is weak and the probability for hookworm carriage in the peri-urban wild dog population is wide spread. The lack of spatial variation is potentially dependent on the survival of hookworm larvae in the environment, the lack of climatic variability within the study area as well as the likely widespread presence of paratenic

hosts and infected bitches (for transmammary transmission). Hookworm eggs are shed into the environment where they develop into the infective larvae stage (L3) of their lifecycle. During the larval development phase, the hookworms have limited mobility and hence their growth and rate of development is highly dependent on surrounding physical environmental and climatic factors (Brooker et al., 2004). Cold temperatures may either delay development of eggs, or cause death (Salih, 1981). On the other hand, temperatures in excess of 37 °C can also inactivate eggs or larval stages. In foxes tested from Western Australia, hookworm prevalence was positively associated with areas with increased humidity, warmer minimum temperatures and increased native vegetation (Dybing et al., 2013). The difference in hookworm carriage reported from this Thesis in Chapter 3 and those of Brown and Copeman (2003); Dybing et al. (2013); and Smout et al. (2013) could be explained by variation in the general climate of southern Queensland (sub-tropical), northern Queensland (tropical) and south west Western Australia (temperate). The climatic variables tested in this study suggested that maximum rainfall was marginally significant ($p=0.074$) and other climate variables were not associated with hookworm carriage in the peri-urban wild dog population. However within the study area there was a lack of major variability amongst the climate variables. Alternatively, clustering may be influenced by density of animals, where wild dogs located in close proximity to each other may result in a higher number of infective larvae in the soil, relating to higher prevalence in the dog population. However, without information on wild dog density, which is unavailable and difficult to collect, this remains an assumption. The weak propensity for clustering reported in this study follows trends that have previously been documented in human hookworm cases, where other soil transmitted helminths have shown significant propensity for clustering, hookworm has not (Soares Magalhaes et al., 2015).

This is the first study to present a predictive map of the probability of hookworm carriage in peri-urban wild dogs in Australia. The high risk clusters, where risk of infection is significant, are primarily located within the southern half of the study area. The detected high probability of hookworm carriage in the southern half of the Brisbane City Council local government area is an important finding. Reasons for such high probability of hookworm carriage in this area is unknown, however, this region has high human density and its highly urban location makes management of wild dogs difficult. These areas with high predicted probability of hookworm carriage are also represented by low to moderate uncertainty, suggesting confidence in the prediction. Areas of low predicted probability of hookworm carriage correspond with high predicted uncertainty, suggesting that these locations require further investigation. Overlap between the hookworm and *E. granulosus* prediction maps suggest that areas of high predicted hookworm carriage correspond with regions of low to moderate *E. granulosus* prevalence. These regions of overlap present significant public

health risk because the low to moderate probability of *E. granulosus* carriage can still represent up to a predicted 55% probability of carriage. An integrated program that controls both parasites in the regions of high predicted hookworm probability could be beneficial.

The predictive map of hookworm carriage can be utilised to inform targeted control of hookworm carriage in wild dog populations in close proximity to human habitation as well as to inform integrated treatment interventions for other parasites carried by wild dogs within the same high risk areas. This is in contrast to methods used in rural areas where a ‘nil-tenure’ approach is applied across the landscape to achieve an overall reduction in population (Hunt, 2005). However, the high predicted probability of hookworm carriage within the southern half of the Brisbane City Council region still provides problems for urban pest management officers. Wild dogs are known to persist in high risk urban environments (McNeill et al., 2016), where traditional methods of control cannot be implemented due to restrictions with toxin use (APVMA, 2008) and limitations on the use of firearms. Recent developments, including new tools for toxin release such as the canid pest ejector, and the release of new toxins such as para-aminopropiophenone (PAPP), are increasing our capacity for control of wild dogs in urban and peri-urban areas (Gentle et al., 2017). However, the prediction map can also assist with education of the general public to ensure that in areas of high predicted probability of hookworm carriage, domestic dogs are dewormed regularly and owners ensure hygienic pick up of their dogs’ faeces in public areas and that people wear shoes in open areas. Full exploration of the public health risk of our findings would require linking prevalence information with data on contact patterns between humans and areas contaminated with wild dogs. Recent GPS collaring data of peri-urban wild dogs within our study area suggests there is spatial overlap between wild dog movement and human associated areas (McNeill et al., 2016). Therefore deworming of domestic dogs and owner education are extremely important. Educating adults on the transmission routes of hookworm (percutaneous infection) and appropriate hygiene (no bare feet in parklands and hand washing after children playing) as well as the clinical signs in humans (pruritic self-resolving rash, abdominal signs) is likely to contribute significantly to our ability to mitigate the potential risk of hookworm infections posed by wild dogs.

The findings of this study need to be interpreted in light of its limitations. As mentioned in previous chapters, the ability to sample across all landscapes was limited due to the opportunistic reliance on wild dog captures from current management programs. Locations of captures were often influenced by public complaints and / or where current proactive management programs were being conducted. Although best efforts were made by the trappers and researchers, collection of faecal material was not always possible at the capture site. As a result, not all cadavers had an associated faecal sample

for flotation and / or PCR. The ecological nature of this study utilised mean values as environmental proxies to measure wild dog exposure to the climatic variables. Hence, the measured effect sizes may result in regression dilution bias which is likely to cause an underestimation of the observed climatic effects (Hutcheon et al., 2010). The observed presence of hookworm showed a lack of spatial autocorrelation, limiting a geostatistical modelling approach, as was applied in Chapter 5. However the application of the regression equation in ArcGIS based on the non-spatial multivariable model successfully produced an informative and useful prediction map of prevalence. One physical environment factor we did not account for in our analyses was soil type. Sandy soils have previously been found to be positively correlated with the presence of hookworm (Mabaso et al., 2003) and the influence of rainfall can be dependent on the ability of the soil to retain water (Smith, 1990). However, it is most likely that the climatic variables within the sampled area did not vary enough to significantly detect climatic risk factors.

Conclusion

This study indicates that the presence of hookworm in wild dogs remains a significant public health concern in peri-urban environments. Hookworm prevalence in the peri-urban wild dog population was not associated with physical environmental factors, however, climatic variables were important confounders in the regression model and maximum rain was marginally significant. High risk regions of hookworm prevalence are mostly located within the southern half of the study area, with a significantly high endemic region in the south of the Brisbane City Council local government area. These locations can be targeted during management programs, not only with peri-urban wild dog control but also including the implementation of public education within high risk regions. Continual monitoring of hookworms both within the peri-urban wild dog population and the environment in the regions of high predicted probability may remain an important factor in understanding the potential routes of human infection. This data, combined with knowledge of other parasites of concern, such as *E. granulosus*, allow for the delivery of an integrated control approach in regions of parasite overlap.

CHAPTER 7

General Discussion

AIMS OF THE THESIS REVISITED

Peri-urban wild dogs often go unrecognised amongst the public because of their limited economic impacts on industries within urban regions. Within urban regions, wild dogs utilise smaller home-ranges than their rural counterparts, and are able to reside within high risk and densely populated regions (Allen et al., 2013; McNeill et al., 2016). Wild dogs frequently traverse human associated environments and are known to carry a variety of infectious pathogens (Mackenstedt et al., 2015). However, information regarding the carriage of zoonotic pathogens in peri-urban wild dogs is limited to few studies with small geographical range and / or small sample size (Allen, 2006; Brown and Copeman, 2003; Jenkins et al., 2008; Smout et al., 2016; Smout et al., 2013). Despite recognising the ability of peri-urban wild dogs to carry zoonotic pathogens, in depth epidemiological studies are lacking. The research presented in this Thesis is the first comprehensive survey of zoonotic pathogens in peri-urban wild dogs in Australia. The survey of pathogens and simultaneous collection of individual level data of each wild dog, along with environmental covariates, has resulted in a unique data set enabling detailed analyses of potential risk factors associated with pathogen carriage. The association between diet and pathogen carriage has been supplemented with an ecological analysis of the role of climate, physical environment, and individual level risk factors in the probability of carriage of the most common and significant zoonotic pathogens. Model-based geostatistics were applied to develop a predictive map of *E. granulosus* carriage and used a logistic regression model to predict areas of hookworm carriage in South East Queensland. The results from this Thesis provide invaluable baseline data on pathogen presence and prevalence, and identified the associated risk factors in peri-urban wild dog populations. Importantly, the outputs will guide future research but will ultimately assist land managers in targeting their management programs towards areas or animals of most significance.

Aim 1: Status of zoonotic pathogen carriage in peri-urban wild dogs (Chapter 3)

At the commencement of this project, there was limited information available regarding zoonotic parasites in peri-urban wild dogs, and as a result, one of the key aims of this study was to survey and provide essential baseline data to close this knowledge gap. Several local governments across south-east Queensland have active peri-urban wild dog management programs which provided the opportunity to collect carcasses for our pathogen survey across this landscape. Peri-urban wild dogs were sampled and tested utilising a number of morphological, microbiological and molecular methods to detect the targeted pathogens. Helminth infections were most commonly identified in peri-urban wild dogs, with *E. granulosus* and *Ancylostoma caninum* as most prevalent. Bacterial pathogens were also detected, however, at much lower prevalence than parasites. The analysis pipeline outlined in this chapter demonstrates that wild-dog carcasses routinely collected by local

government areas can constitute an essential source of surveillance data that can be used to monitor temporal trends in pathogen prevalence in peri-urban wild dog populations, and allow for monitoring geographical trends across different environments. This can be used to inform the management of peri-urban wild dogs in Australia.

Aim 2: The association between diet and the presence of pathogens (Chapter 4)

Knowledge of wild dog diet has traditionally been reliant on the identification of food items in scats, which may underrepresent the consumption of readily-digestible food items. Information regarding peri-urban wild dog diet is also limited, with the first formally published report by Allen et al. (2016). The unique opportunity to collect wild dog stomachs whilst sampling for pathogens enabled an assessment of the association between dietary composition and pathogen presence. Diet influences host-pathogen interactions in several wildlife species around the world and this study was no different, demonstrating that the consumption of swamp wallaby and other macropods was positively associated with the presence of *E. granulosus* in peri-urban wild dogs. The presence of bandicoot and birds within the stomachs of wild dogs were also shown to be positively associated with the presence of hookworm infection. The limited number of dogs testing positive for targeted bacteria restricted the opportunity for formal statistical analyses of a dietary pathway for zoonotic bacterial carriage, however a greater percentage of dogs with vegetative matter in their stomachs were found to be positive for either *Salmonella* spp., *Staphylococcus aureus*, or *Escherichia coli*. This chapter revealed the importance of managing both the definitive and intermediate stages of parasites for effective control of pathogens in wildlife. Evidence presented in this chapter enhances existing knowledge of factors that drive disease which supports the identification and implementation of strategies to manage the currently uncontrolled pathogens carried by peri-urban wild dogs.

Aim 3: Spatial variation and risk factors associated with pathogen prevalence (Chapters 5 and 6)

Understanding drivers of disease provides knowledge that can assist in mitigating impacts and risks through the implementation of strategic management programs. To understand which risk factors influence the presence of pathogens in peri-urban wild dogs, infection status was compared to individual level factors (age and sex) for each wild dog along with a range of physical environment and climate variables extracted utilising geographical information systems. The prevalence and spatial distribution of two pathogens, *E. granulosus* and hookworms, were assessed for their relationships with associated individual level, physical environment and climate risk factors. Pathogens that were foci of the analyses in Chapters 5 and 6 were selected based on their prevalence in dogs (Chapter 3), the known potential for severe disease in humans, and the

availability of treatment in humans. Although *Spirometra erinacei* had a higher detected prevalence in the sampled peri-urban wild dog population, the potential implication for human disease is considered to be less than that presented by hookworms.

Geographical distribution and ecological determinants of the probability for Echinococcus granulosus carriage in wild dogs (Chapter 5)

Our results demonstrated that the probability of *E. granulosus* carriage was not associated with age or sex differences of peri-urban wild dogs; however intensity of infection was higher in bitches and pups under six months old compared to male and wild dogs above 6 months old, respectively. By applying the model-based geostatistical model, a predictive map of the probability of *E. granulosus* carriage by peri-urban wild dogs was generated. Climate and the physical environment were not associated with the presence of *E. granulosus*, however, they did account for the majority of the spatial dependence within the data. The predictive map highlighted areas of high endemicity (with low uncertainty) within the southern region of the Gold Coast City Council, the Byron Bay Regional Council, and a small section of the south-west corner of the Sunshine Coast local government area. This map indicates priority areas to implement peri-urban wild dog management programs to target areas where *E. granulosus* carriage is predicted to be high, and hence, potentially reducing the risk of transmission to humans. The detection of significant clustering confirms that a broad scale management protocol, as commonly used within rural regions, is not the best approach within the peri-urban environment. This is extremely beneficial information for land management and can assist to efficiently and effectively mitigate the impacts of peri-urban wild dogs in these regions.

Geographical distribution and ecological determinants of the probability of hookworm carriage in wild dogs (Chapter 6)

The carriage of hookworms was not associated with sex, age, or physical environmental factors, however, maximum rainfall was moderately significant. Unlike *E. granulosus*, the spatial variation of hookworm was not significant and suggested that its propensity for clustering was very weak. As a result, the probability of hookworm carriage was modelled using a regression equation in a geographical information system. This revealed highly endemic regions of hookworm in the southern half of the Brisbane City Council local government area and also in the south western region of the study area. Together with Chapter 5, the results of both the prediction maps provide tools for identifying and developing strategies for mitigating public health risks associated with peri-urban wild dogs. Wild dog management in peri-urban regions still remains a difficult challenge due to the limitations on methods for control and risks towards domestic dogs. The prediction maps

presented in this Thesis can assist management programs to best allocate their limited resources to implement targeted programs within the regions of high endemicity thus potentially enhancing efficacy of existing programs.

CAN PERI-URBAN WILD DOGS POSE A ZOOTIC DISEASE RISK TO HUMAN COMMUNITIES?

To address the main aims of the Thesis, four component questions have been proposed that together can help answer the overarching research question. These four component questions addressed each Thesis objective in turn, and reconciled the information from the research chapters to provide the framework for further discussion of the public health implications of wild dogs within peri-urban regions.

1. Can wild dogs be infected with zoonotic pathogens and to what degree?

The findings in Chapter 3 demonstrate that peri-urban wild dogs are infected with pathogens that can be transmitted to humans. Pathogens detected include parasitic and bacterial species, however, further research to identify potentially zoonotic viral pathogens that peri-urban wild dogs may be exposed to, for example Hendra virus and bat lyssavirus, is required. Wild dogs also have the potential to carry and disseminate emerging diseases. Although not detected in this study, they could pose as sources for pathogens, including *Brucella suis*. Considering the prevalence of the detected pathogens in peri-urban wild dogs and their potential for causing disease in humans, the most significant pathogen carried amongst the peri-urban wild dog population is the tapeworm *E. granulosus*. *E. granulosus* was present amongst just over 50% of the wild dog population sampled. The ability of infective eggs to travel and spread via wind, flies and within soil via accidental hosts and fomites (e.g. on shoes) enhances the range of the parasite. This is a significant public health concern, considering these animals regularly reside within or a close distance from human settlement. The high prevalence of hookworms carried by peri-urban wild dogs presents risk to humans, particularly children, playing barefoot in playgrounds, parks, sporting fields and sandpits. However, not all pathogens are present in such high prevalence and not all pathogens have as serious implications on human health. *Toxocara canis* was only detected in 5.4% of the peri-urban wild dog population sampled. This low prevalence in the wild dog population suggests that there may be a lack of infective eggs within the environment, and hence the opportunities for human infection would be limited. Despite this, eggs are long lasting and can build up in the environment. Hence, wild dog infection with *T. canis* remains important due to the implications of *T. canis* infection in humans and especially in younger children. Continued monitoring of the presence of this pathogen within peri-urban wild dog populations, as well as monitoring co-located human

infection rates, should remain a priority. Other pathogens such as *Spirometra erinacei*, although highly prevalent within the wild dog population, do not pose direct risk to human health and are considered to be a low priority for future monitoring or management.

2. What are the likely sources of infection?

It is likely the most common source of infection for peri-urban wild dogs is through direct consumption of infected material in their diet. The research in Chapter 4 of this Thesis combined with a larger scat survey across Queensland has revealed that peri-urban wild dogs do not frequently utilise anthropogenic sources of food and rely on natural sources of prey. Although the information provided from stomach contents provides a short ‘snapshot’ of diet at that time, wild dogs are typically reliant on just one or two species of prey when readily available. The associations between diet and pathogen presence from this study remain epidemiologically significant and can be explained by known characteristics of pathogen lifecycles. Although diet is likely to be a common infection pathway in wild dogs, there are other important routes of transmission that are dependent on the pathogen that is being considered. For example, infection with *Ancylostoma caninum*, the most commonly detected species of hookworm in peri-urban wild dogs, is transmitted both vertically, from bitch to pup via the transmammary route, and horizontally. Although there were no detected age and / or sex factors significant for hookworm prevalence, females were almost twice as likely to have been recorded positive for hookworms than males. When considering the importance of vertical transmission and the likelihood of infection in bitches, this pathway becomes a significant source of hookworm infection for young pups and hence, maintaining infection within the wild dog population. However, there are also potentially several species of paratenic hosts of hookworm within the environment and our data suggests that these may also be a significant factor in the maintenance of the parasite within wild dog populations.

3. What are the key drivers of the level of endemicity?

Age and sex were not associated with of endemicity in both the *E. granulosus* and hookworm datasets. However, it appears that *E. granulosus*-infected females have significantly higher intensity with greater ability to contribute towards environmental contamination according to the Index of Potential Contamination. Dominant bitches can be difficult to remove by trapping (the dominant control technique for peri-urban wild dog populations) and other control techniques are limited in such environs. This could be a significant contributing factor to the ability of the pathogen to survive within the population. Maximum rainfall had a slight negative effect on the probability of hookworm carriage, however there was no detection of any other climatic factors, influencing prevalence. Climatic factors included in our models of *E. granulosus* carriage accounted for the

majority of the geographical clustering of *E. granulosus* carriage. It is still likely that climatic factors play a role, but may be apparent only when examined at larger geographical scale and when measured without resorting to remotely-sensed maps. *E. granulosus* appears to be suited to the sub-tropical climate of southern Queensland, in contrast to the tropical regions of the state (northern Queensland) where it has not been detected. However, carriage of hookworm in wild dogs would be expected to be higher in the tropical climate of northern Queensland compared to southern Queensland. Hence, different pathogens favour different climatic factors which will likely influence prevalence. Within the study area for this project it is possible that the climatic factors that were tested for did not vary enough to detect significance amongst the variables.

4. Can infected dogs inhabit locations in close proximity to human communities?

Recent studies have demonstrated that wild dogs are capable of residing within close proximity of human population centres. However, our understanding of how urbanisation influences the prevalence of pathogens was not well known. The results presented within this Thesis reveal the significant extent of pathogen carriage within a population of peri-urban wild dogs that are known to live within 1 km of human establishments at all times. The prevalence of zoonotic pathogens in peri-urban wild dogs that are known to traverse through public parklands, school grounds and other suburban regions that are utilised by families for recreation purposes is now better understood. The carriage of zoonotic pathogens should be considered in future peri-urban wild dog management programs. The developed predictive maps for probability of *E. granulosus* and hookworm carriage has demonstrated that high endemicity zones are located within urbanised landscapes across south-east Queensland and northern New South Wales. These data confirm that wild dogs infected with zoonotic pathogens inhabit locations within close proximity to human communities.

LIMITATIONS

The main limitation of this study was the use of convenience samples. Utilising samples passively collected through ongoing local government wild dog management programs was the optimal and most ethical method to quickly sample across a large geographic area, and to capture the full breadth of pathogens possibly present. The logistics of cadaver transport and storage that were accessible to trappers was a limiting factor in the contribution of wild dog study numbers. The management of field collected samples was also a challenge, especially the collection and storage of whole blood and serum by trappers. It is often impractical to immediately prepare or refrigerate serum samples, leading to a reduced number of viable blood and serum samples. The timing of animal capture is largely unpredictable, and it can take several hours to check traps each day.

There were several limitations that required the elimination of some pathogens of interest from the screening strategy for this study. Some pathogens were forcibly excluded based on a combination of risk group level (and access to suitably certified laboratories), access to positive controls, and limitations for testing in Australia. Some pathogens that were included on the initial list for testing included Australia Bat Lyssa Virus (ABLV) and Hendra Virus. Both viruses have considerable public health implications, particularly in Queensland, and the role of dogs in the transmission of these agents is unclear. However, the emergence of either ABLV or Hendra within the wild dog population would have substantial consequences and it reiterates the need for effective surveillance of emerging or potentially emerging diseases in wildlife populations. Pathogens such as *Brucella suis* were of significant interest due to the recent discovery of several pig-hunting dogs in NSW presenting with the disease (Mor et al., 2016). Although testing was performed for *B. suis*, the limited number of serum samples that were collected and sample quality significantly limited our ability to detect the pathogen within the wild dog populations. Future studies that focus on the detection of some unlikely but significant pathogens would provide valuable contributions to our knowledge of disease epidemiology in the peri-urban wild dog populations. This would require a different sampling and screening strategy than the one used for the current study, which was more interested in estimating the prevalence of carriage of more common pathogens. The ecological aspect of the final two research chapters in this Thesis may be prone to regression dilution bias. Mean values were used as environmental proxies to measure wild dog exposure to climatic variables. It is possible that the measured effect sizes represent an underestimation of the observed climatic effects.

FUTURE RESEARCH IN AUSTRALIA

This project has investigated zoonotic pathogens carried by peri-urban wild dogs and significantly adds to the knowledge of wild dog ecology in Australia in a number of ways. Firstly, the data presented within this Thesis provides the foundation for future investigations into the potential for peri-urban wild dogs to act as a reservoir for bacterial and viral pathogens. However there is further need for more research relating to the most significant emerging public health threat of drug-resistant bacteria. Wildlife populations that carry bacteria resistant to one or several antimicrobials proves a significant risk to human health as it enables to the spread of resistance genes and bacteria into the environment and to other species of animals. Understanding the role of peri-urban wild dogs in the transmission of multi-drug resistant bacteria will improve knowledge surrounding aspects of disease epidemiology, general wild dog ecology, and how this may impact management strategies for control. However, most screening methods for zoonotic pathogens carried by peri-urban wild dogs are labour intensive (e.g. dog trapping and necropsy). Research into practical and

applicable screening methods from convenience samples such as scats, or diagnostic tests that can be conducted at the time of trapping, would significantly improve access to information.

Secondly, one of the major outputs from this Thesis is the development of predictive maps of the probability of hookworm and *E. granulosus* carriage by peri-urban wild dogs. This information could be utilised as spatial decision-support tools to help conduct soil testing for viable eggs or larvae in the environment across different urban areas (assisted by GPS tracking data and predicted high / low prevalence areas). This would enable us to verify the prediction maps and determine if pathogen levels in the soil correspond with the predicted / actual prevalence carried by wild dogs. Understanding the prevalence levels in wild dogs and their association with environmental contamination will allow for a broader understanding of how these pathogens are surviving within the environment. Another application of the prediction maps of hookworm and *E. granulosus* carriage is for future investigations into the implementation of control programs. This has not only provided a tool for management of the impacts of peri-urban wild dogs, but provides a starting point for measures to be recorded in regards to success of management.

Control programs may include either targeted wild dog population reduction in high risk zones or could include baiting trials with praziquantel. Baiting with anthelmintics is a method implemented in the northern hemisphere where urban foxes are definitive hosts of the tapeworm *Echinococcus multilocularis* (Hegglin and Deplazes, 2008). Baiting has seen significant reductions in the presence of the parasite within the fox populations only when it has been applied in defined risk areas, not across large landscapes (Hegglin et al., 2003). The prediction map of *E. granulosus* prevalence would enable trials of praziquantel baiting within predicted high risk zones. However, our data presented in Chapter 5 suggests that this would be best achieved with combined and continued monitoring of both wild dogs for adult *E. granulosus* and macropods for the presence of hydatid cysts. This would enable investigations to be made in the success of baiting across both the definitive and intermediate stages of the parasite. Neuter and release programs could influence parasite prevalence because both estrogen and testosterone are immunosuppressive (Klein, 2004). However, trapping peri-urban wild dogs remains labour intensive and many council management programs operate on the basis of response to public complaints of wild dog impacts. Research within an enclosed facility to trial the impact of sterilisation on parasite carriage by wild dogs would be necessary before implementing a program in the field.

Some aspects of this research have been limited by the lack of specific knowledge surrounding peri-urban wild dog densities and also densities of prey species. Any advancement that contribute

towards developing techniques to monitor wild dog and prey species density, and ecological aspects of peri-urban wild dog biology in general will assist with better understanding their role in the transmission of zoonotic pathogens.

PRACTICAL IMPACTS AND RECOMMENDATIONS

The current control methods for peri-urban wild dogs are not likely to be sufficient for managing the impacts of zoonotic pathogens. Hence, it is important to continually monitor wild dogs for the type and prevalence of infectious pathogens that they carry, and to be aware of measures that can be taken to manage the presence of zoonotic pathogens within the peri-urban wild dog population.

The highest risk group within peri-urban wild dogs for infection with *E. granulosus* is females aged less than six months old. Wild dog management programs should prioritise targeted control of den sites within the predicted high risk areas identified in this Thesis. Den sites may be best targeted with intensive trapping regimes when pups start to become independent of their parents and begin to expand their geographical home range. The implementation of anthelmintic baiting trials could be conducted in conjunction with other control methods. Baiting with praziquantel has previously been shown to be successful in reducing the prevalence of *E. multilocularis* in foxes in other regions of the world (Hegglin et al., 2003; Takahashi et al., 2013). The nature of the anthelmintic requires continued management throughout the year. Research trials would need to be conducted to determine an appropriate baiting schedule, and the number of baits to be laid per square kilometre. The highest risk group within peri-urban wild dogs for hookworm carriage are females aged one to two years old. Targeted control may not be able to focus on this particular sex and / or age combination however, management programs can be directed to geographical locations of predicted high pathogen carriage.

Geographical regions of predicted high hookworm carriage in peri-urban wild dogs overlap with areas of low to moderate predicted *E. granulosus* carriage. This suggests that to manage the presence of hookworm within the peri-urban wild dog population, baiting with anthelmintics should be targeted to reduce the presence of both pathogens in those regions. This approach is to target both species of parasites, because of the potential implications towards public health. The probability of *E. granulosus* carriage in low to moderate predicted regions can be as high as 55 percent. Hence, integrating control to target both pathogens within these regions where hookworm is predicted to be the dominant parasite could be beneficial to reduce the overall presence of zoonotic pathogens carried by peri-urban wild dogs. However, the feasibility of implementing such a management program is unknown, and research into the viability of a combined program would

be required. In comparison, geographical regions of predicted high *E. granulosus* carriage corresponded to low predicted probability of hookworm. Programs could specifically employ protocols to solely target a reduced presence of *E. granulosus* in these regions. Again, management programs are likely best achieved through a combination of targeted trapping and continual baiting (with anthelmintic) programs. To implement successful management programs to control zoonotic pathogens it is important to first identify the regions of predicted high and low probability before implementing targeted control methods.

Lastly, public education in all peri-urban and urban landscapes is important to assist with mitigating the potential spread of pathogens into human populations, and their impact thereafter. Children should avoid walking in public parklands with bare feet, avoid eating soil, and always wash hands after playing. Education of trappers and those involved in management programs is also a significant factor in mitigating disease transmission from wild dog populations to humans as many pathogens can be prevented by the implementation of proper hygiene protocols. The development of a guide detailing appropriate personal protective measures when handling wild dog cadavers would assist landholders and others involved with wild dog management to protect themselves against pathogens of concern, in particular *E. granulosus*. Main areas of discussion should include the use of personal protective equipment, how to appropriately dispose of (or wash) used personal protective equipment, and how to dispose of wild dog or other feral animal cadavers. Significant focus should be placed on the importance of hand washing prior to the consumption of food items. The use of disposable gloves when handling wild dogs, and the implementation of appropriate hygiene protocols are simple and major preventatives for human infection.

CONCLUSION

Wild dogs have an important role in the maintenance of zoonotic pathogens within peri-urban environments. The landscape use and home-ranges of peri-urban wild dogs allows for a direct link, through the environment or through pathogen exchange with domestic dogs, for the transmission of zoonotic pathogens to humans. Management programs within these peri-urban zones are best implemented towards targeted areas, in comparison to population level reductions across the landscape as utilised in rural regions. The prediction maps can be applied by land managers to target areas of high predicted probability of pathogen carriage and can be utilised for monitoring the presence of pathogens in future surveys. The results of this Thesis stress the significance of public education in mitigating risks towards public health. The combined efforts of awareness of health in domestic dogs (e.g. correct deworming protocols), and communication of the importance of hand

washing, particularly prior to food consumption, can assist in mitigating the spread of zoonotic pathogens carried by the peri-urban wild dog population.

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